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Incidence rates of prostate cancer are higher in blacks than in any other racial group. Our laboratory is attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer in blacks. It is hypothesized that Africans may have genetically down-regulated their zinc absorption capacity; otherwise, they would absorb abnormally high levels of zinc, resulting in various serious neurodegenerative disorders. We hypothesized that people of African origin may have a lower capacity to absorb zinc when compared with other racial groups because of their inherent down-regulation of zinc transporters. This notion was tested by evaluating 58 prostate cancer tissues in 2 major racial groups (30 from whites and from blacks) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from white people, the degree of expression of these 2 zinc receptors was high when compared with age-matched specimens obtained from blacks. These data are being confirmed in larger groups by utilizing zinc indicators that measure the actual intracellular zinc levels in the prostate tissues. This finding could have significant application as a preventive maneuver for at least some people. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

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Table of Contents

Introduction

Why are African American men, after 10 to 20 generations of residing in the U.S., twice as likely to develop cancer of the prostate as Caucasian Americans, while South African Blacks are 10 to 30 times less likely to develop prostate cancer than their American distant cousins, and 2 to 10 times less likely than African Whites presumed to have a mixed European ancestry somewhat similar to that of White Americans? Are there any consistent differences in the expression of significant genes or proteins in the prostate cancers taken from African Americans versus those from White Americans? A study of the genes and proteins which influence the expression of any gene confirmed to be disparately expressed might lead to the identification of one or more environmental or living pattern factors worthy of epidemiological research for its potential relationship to the incidence or progression of prostate cancer in American Blacks, Whites, or other ethnic groups.

In this research project, it is our goal to test this hypothesis by analyzing prostate cancer tissues for the expression of mRNAs that code for various zinc transporter proteins. Our goals are to: 1) determine the expression levels of all three zinc *transporters (hZIP1, hZIP2* and *hZIP3*) in the neoplastic prostates from African Americans verses Whites by utilizing the quantitative reverse-transcriptase (RT) polymerase chain reaction PCR (RT-PCR), RT *in situ*-PCR, and immunocytochemistry methods, 2) measure the expression levels of all three zinc transporters (*hZIP1, hZIP2* and *hZIP3*) in the normal prostatic tissues from African Americans verses Whites, 3) measure the intracellular zinc levels and the location of zinc inside the various cell types that make up the cancer and normal prostate tissues, and examine in a few specimens the gene sequences of zinc transporters and their promoters, which presumably regulate the degree of expression of these genes, and 4) evaluate the zinc, testosterone and prolactin levels in the blood samples of over 2,000 individuals from all major races, especially in African American verses Whites. If a direct or environmental link between Zinc transport and prostate cancer can be established, then a special nutritional formula, medication, or other intervention might be especially designed to test the ability to decrease the incidence of this disease in African Americans. Such an intervention, if successful, might be useful for persons of all populations.

In the United States, prostate cancer is the most commonly diagnosed male cancer and the second leading cause of all male cancer deaths. African-Americans have the highest prostate cancer incidence rates in the world. Our laboratories are attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer, with special emphasis on the disproportionately high incidence rates in African-Americans. It is hypothesized that since Africa is a mineral-rich continent and the zinc levels in the water and diet are very high, Africans may have genetically down-regulated their zinc absorption capacity; otherwise they would absorb abnormally higher levels of zinc, which reportedly results in serious neurodegenerative and biochemical disorders. Therefore, individuals of African origin may have a lower capacity to absorb zinc due to their inherent down- regulation of zinc transporters when compared to other racial groups. Extensive research has shown that the low serum levels of zinc have been associated with the increased incidence of prostate cancer. Our laboratories have been collaborating with the Cleveland Clinic Foundation, the Medical College of Wisconsin, and the Medical Examiner's Office of the State of Maryland to determine the degrees of expression of various zinc transporters at the molecular level. Therefore, we have evaluated 58 prostate cancer tissue samples in 2 major racial groups (30 from Caucasians and 28 from African-Americans) for their ability to express two major human zinc transporters, hZIP1 and hZIP2. In all of the 30 prostate cancer specimens obtained from Caucasian individuals, the degree of expression of these two zinc receptors was higher when compared to age matched and the tumor grade level score matched specimens obtained from African-American patients. We also found significant down-regulation of these two zinc transporters in normal prostate tissues from African-American men as compared to age matched Caucasian men. When compared with normal prostate tissues, the expression levels of the zinc transporters were relatively lower in the neoplastic tissues from both racial groups tested. The loss of a unique capability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. However, there are several questions that need to be answered before a firm correlation can be established between the actual intracellular zinc levels in the prostate glands of various racial groups and the incidence rate of prostatic neoplasm. In addition, a causal relationship needs to be determined between the zinc levels in the prostate and the levels of expression of zinc transporters, in situ. Therefore, in order to answer these questions at the molecular and cellular levels, our goals are to examine the prostate tissues and sera from the corresponding patients and explore the following **Specific Aims**:

1) To determine the expression levels of all three zinc *transporters (hZIP1, hZIP2* and *hZIP3*) in the neoplastic prostates from African Americans verses Whites, by utilizing the quantitative reverse-transcriptase (RT) polymerase chain reaction PCR (RT-PCR) method, RT *in situ*-PCR, and immunocytochemistry (Completed).

2) To measure the expression levels of all three zinc transporters (*hZIP1, hZIP2* and *hZIP3*) in the normal prostate tissues from African Americans verses Whites (Completed).

3) To measure the blood zinc levels in about 2,000 individuals and compare the serum zinc levels in African-Americans, Africans, Caucasians, Asians and mixed racial groups. These studies will be performed at the Principal Investigator's site-Claflin University--a HBCU. For this purpose, the majority of the serum specimens will be obtained from the Medical University of South Carolina (MUSC). In addition to zinc levels, we will also measure the testosterone and prolactin levels in these groups (data are being collected and analyses).

4) In a separate but smaller group of individuals, autometallography and atomic absorption spectrophotometry will be used to measure intracellular zinc in various anatomic parts of the prostate tissues (zinc indicators are being utilized to measure the intracellular levels of zinc in situ: This method is far more superior than what was proposed: partially completed).

5) To determine which other factors, including the exposure to prolactin, testosterone, external zinc concentrations, and combinations of these three agents regulate the zinc transporters in the pre-established prostatic cell lines (i.e. PC-3 and La cell lines) and primary cell lines established from the prostate tumors from various racial groups. We believe that by establishing a link between the low intracellular transport capacity of zinc in the African-American population and development of prostate cancer, we may be able to design protocols which can increase intracellular zinc levels in the prostate gland. In addition, we hope to identify certain unique genes that may be selectively expressed or suppressed in certain racial groups. These studies may also shed some light on why men from all races develop prostate cancer in old age and

how it is linked to intracellular zinc levels and serum zinc, testosterone, and prolactin levels (this work is in progress: hope to be completed by the end of March 2005).

Body

PROGERESS IN THE LAST 24 Months

AIMS 1-2:

To determine the expression levels of all three zinc *transporters (hZIP1, hZIP2* and *hZIP3*) in the neoplastic prostates from African Americans verses Whites, by utilizing the quantitative reverse-transcriptase (RT) polymerase chain reaction PCR (RT-PCR) method, RT *in situ*-PCR, and immunocytochemistry.

To measure the expression levels of all three zinc transporters (*hZIP1, hZIP2* and *hZIP3*) in the normal prostate tissues from African Americans verses Whites.

We are pleased to inform the agency that we have completed the AIMS #1-2 of the project and published an article (attached as Appendix 1). The main points of our data are described below.

ABSTRACT: As it is mentioned above that in the United States, prostate cancer is the most commonly diagnosed male cancer and the second leading cause of all male cancer deaths. Furthermore, incidence rates are higher in African Americans than in any other racial group. Our laboratory is attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer in African Americans. Because Africa is a mineral-rich continent, and the zinc levels in the water and diet are high, it is hypothesized that Africans may have genetically down-regulated their zinc absorption capacity; otherwise, they would absorb abnormally high levels of zinc, resulting in various serious neurodegenerative and biochemical disorders. It is therefore possible that people of African origin may have a lower capacity to absorb zinc when compared with other racial groups because of their inherent down-regulation of zinc transporters. This notion is further supported by our new initiatives in the areas of diabetes mellitus, hypertension, cardiovascular disease, and pancreatic cancer, incidence of which are higher in African Americans appears to be linked to zinc transporters. Extensive research has shown that low serum levels of zinc are associated with the increased incidence of prostate cancer. We have evaluated 58 prostate cancer tissues in 2 major racial groups (30 from whites and 28 from African Americans) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from white people, the degree of expression of these 2 zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients. We also found a significant down-regulation of these two zinc transporters in normal prostate tissues from African American men when compared with age-matched White men. The loss of the unique ability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. Our observation that the uptake of zinc may be different in racial groups is intriguing and relevant. Once these data are confirmed in larger groups, this finding could have significant application as a preventive maneuver for at least for some people. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

Details of the Results: The prostate contains high amounts of free zinc ions that are excreted into the seminal fluid. The extracellular and intracellular distribution of zinc ions in the rodent using highly specific autometallographical studies have shown that zinc accumulates primarily in the acinic lumen of the lateral lobes, whereas the dorsal lobe stains only faintly and the ventral lobe is void of grains (1, 2). At the ultrastructural levels, the presence of zinc ions is confined to apical secretory vesicles and the epithelium of mainly the lateral lobes in both rodents and humans (1, 3). Recently, Iguchi et al, using semiquantitative reversetranscription polymerase chain reactions (SQ-RT-PCRs), showed that the expression of zinc transporters (ZnT) in rats was very high in the lateral and dorsal prostate and much lower in the ventral prostate. In humans, it appears that the zinc ions are constantly secreted from the epithelial cells into both the acinic lumen and the intercellular canaliculi (5). Prostate secretory epithelial cells have the unique function and capability of accumulating extremely high intracellular levels of zinc (2–5). One of the effects of this accumulation is the inhibition of cell growth, partly because of an increase in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial apoptogenesis (6). Prolactin and testosterone regulate zinc accumulation in the prostate; however, little information is available concerning the mechanisms associated with zinc accumulation and its regulation in prostate epithelial cells (7, 8). By using the human malignant prostate cell lines LNCaP and PC-3, Costello et al (7) have shown that the zinc accumulation in both cell types is stimulated by physiologic concentrations of prolactin and testosterone. Their studies reveal that these cells possess the ability to uptake zinc rapidly, indicative of the presence of a plasma membrane high-affinity zinc transporter, possibly by the regulation of the ZIP-type zinc transporter gene expression (7, 8). Kinetic studies demonstrate that the rapid uptake of zinc is effective under physiologic conditions that reflect the total and mobile zinc levels in circulation (8). Correspondingly, genetic studies demonstrate the expression of a ZIP family zinc uptake transporter in both LNCaP and PC-3 cells (8). Some of these zinc-accumulating characteristics are found to be specific for prostate cells. These studies support the concept that prostate cells express a unique hormone-responsive, plasma membrane-associated, rapid zinc uptake transporter gene that is associated with their unique ability to accumulate high zinc levels (9–11). In the United States, the incidence of prostate cancer is significantly higher in African Americans than in White people or Asian Americans (12–19). We hypothesized that because Africa is a mineral-rich continent and zinc levels are relatively high in the water and diet, abnormally high amounts of zinc in the blood may result in various neurological and metabolic abnormalities. The zinc absorption and transport systems are genetically down regulated in the African populations. The phenomenon may be similar to sickle cell anemia, in which a single mutation has provided the survival advantages against the ravages of the fatal form of malaria (20). We hypothesize that when African people entered North America mostly during the slave trade, they encountered an environmental problem: in North America, the zinc levels are relatively low, and the native populations (the American Indians) and other races who migrated from Europe and Asia have a higher capacity to transport zinc to various organs, especially to the prostate gland, in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in the White and Black populations (21). Here the situation is reversed. The White population is unable to up-regulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect them from damaging solar ultraviolet (UV) light. Squamous cell carcinoma is the most common tumor of sun exposed epithelium in White populations (21). Therefore, just as White people carry an evolutionary disadvantage against the solar UV rays outside of their ancestral low-UV light environment, the low absorption capacity of zinc has created a disadvantage in the peoples of African descent who have migrated outside Africa. If this is the case, long-term low serum concentrations of zinc deprive the prostate gland of its essential source of vital trace mineral ingredients, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the idea that low levels of zinc contribute to the high incidence of prostate cancer (1–10) In this research study, it was our goal to test this hypothesis by analyzing the prostate tissues for their ability to express 2 major zinc transporters responsible for the accumulation of zinc in the prostate glands. For this purpose, we used a highly sensitive RT-in situ-PCR method to compare the relative levels of expression of the 2 zinc transporters, *hZIP1* and *hZIP2,* in 2 racial groups in the United States (White and African American).

African Americans have the highest prostate cancer incidence rate in the world (13–18, 25). On a global level, the rates of incidence are low in Asian and African men, low to moderate in White men, and high in African American men (13, 25). Using data collected between 1988 and 1992, Wingo et all reported that African Americans have a 35% higher incidence rate and a 223% higher mortality rate from prostate cancer when compared with Whites.

The differences in the incidence and mortality between African Americans and Whites are attributed to screening, environmental, and biological factors (16, 17). When compared with White controls, Black men present at a younger age with a higher grade and stage of the disease for their age, and with a greater delay in diagnosis (18, 19). Whether the pathogenesis of prostate cancer is different in African American men compared with White men remains unanswered. Whittemore et al (19, 25) note that African American men appear to have a larger volume of "latent prostate cancer." These investigators believe that larger-volume latent carcinomas are those that progress to become clinically evident at a faster rate, suggesting that the events that account for racial differences in prostate cancer incidences may occur very early in cell transformation, and thus may be genetically controlled. We have hypothesized that the major reason for the high incidence of prostate cancer in African Americans may be their inherent inability to absorb or transport normal amounts of zinc from the Northern American environment, in which there is a relatively low concentration of zinc in the diet. We have further hypothesized that zinc absorption and transport systems are genetically down-regulated in some of the African populations. Such natural selection may occur because of the serious adverse effects on the nervous system caused by high zinc levels (26–28). However, when African people entered North America, mostly during the slave trade, they may have encountered an environmental disadvantage because of their inherent down-regulation of zinc transporters. On this continent, the zinc levels are relatively low, and the native populations (the American Indians) and other races that migrated from low zinc areas, i.e., Europe and Asia, have a higher capacity to absorb zinc and are able to transport it to the other organs in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in white and nonwhite populations. The white population is unable to upregulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect themselves from the damaging solar UV light of wavelengths between 290 and 320 nm. Skin cancer is the most common type of cancer in the United States; more than 600,000 cases of skin cancer are reported each year in this country in the white population. Squamous and basal cell carcinomas are the most common tumors of sun-exposed skin areas in this group (21). Just as white people carry an evolutionary disadvantage against the solar UV light outside their low UV light ancestral environment, the low absorption capacity of zinc has created a disadvantage in people of African descent when they migrate outside Africa. Long-term low serum concentration of zinc deprives the prostate gland of its essential source of a vital trace mineral ingredient, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the association of low levels of zinc with prostate neoplasia (3–6). Zinc is an essential nutrient to all organisms because it is a required catalytic or structural cofactor for hundreds of zinc-dependent enzymes and other proteins such as transcription factors. An example of the effect of the low serum levels of zinc can be seen in the animal model of the *lethal milk* mouse mutant (29). ZnT4 is deficient in the *lethal milk* mouse mutant, in which pups of any genotype suckled on homozygous *lethal milk* mothers die of zinc deficiency before weaning. The zinc level in the milk of homozygous *lethal milk* animals is approximately 50% than that of normal animals, demonstrating that ZnT4 plays a crucial role in development (29). Various reports suggest that regardless of race and geographic location, the etiology of certain prostate carcinomas may be linked to zinc transporters. Because the expression of zinc transporters also appears to be regulated by prolactin and testosterone, an age-related increase in the incidence rate of this malignancy may also be indirectly linked to the zinc uptake by the prostate gland (8–11). Members of the *ZIP* family are found in all cellular life forms, including *archaebacteria, eubacteria,* and eukaryotes (10, 30–34). There are currently approximately 85 members reported in the sequence databases. These fall into 4 subfamilies based on their amino acid similarities.10 Most members are predicted to have 8 transmembrane domains and share a predicted topology where the amino and carboxyl termini are extracytoplasmic. There are 12 known ZIP members in the human genome (31). Three of the human proteins, *hZIP1, hZIP2*, and *hZIP3*, are very closely related to the fungal and plant proteins known to be zinc uptake transporters. *hZIP2* expression has been detected only in prostate (31) and uterine (32) epithelial cells, suggesting that this protein plays a very specialized tissue-specific function. On the other hand, hZIP1 is expressed in all 24 human tissues examined so far.33 Gaither and Eide (10, 11, 31) have sequenced and characterized the *hZIP2* gene, a human zinc transporter identified by its similarity to zinc transporters recently characterized in fungi and plants. *hZIP2* is a member of the *ZIP* family of eukaryotic metal ion transporters that includes 2 other human genes, *hZIP1* and *hZIP3*, and the genes in mice and rats (10, 11). The human genome contains at least 3 *ZIP* family members. The current hypothesis is that these genes encode zinc uptake transporters (10, 11). We can gain some insight into *ZIP* function in humans by considering the tissues in which these proteins are expressed. Repeated attempts by Gaither and Eide11 to detect *hZlP2* mRNA on Northern blots of poly (A) + RNAs derived from different human tissues and cultured cell lines failed to produce positive results. It appears that the *hZIP2* transporter gene is normally expressed at low levels and in specific cell types, and that a more sensitive detection method is required. We also attempted to quantitate *hZIP2* mRNA by Northern blots; however, several attempts were not productive. Therefore, we decided to use highly sensitive RT-in situ-PCR and SQ-RT-PCR methods. Gaither and Eide10 isolated only 4 *hZIP2*-expressed sequence tag clones found only in prostate and uterine cDNA libraries. The observation that these particular tissues express *hZIP2* may be instructive in that cells of the prostate contain the highest zinc level of any soft tissue in the body. Any potential down-regulation in this transporter may play a pivotal role in the pathogenesis of prostate cancer. Thus, it appears that the expression of *hZIP2* in prostate and uterine tissues may help meet their particular needs of zinc metabolism. In contrast, the low affinity *hZIP1* and *hZIP3* have been cloned as expressed sequence tags from a large number of different tissues, indicating that these genes are widely expressed and may play general housekeeping roles (10). Therefore, observed zinc transporter expression may be associated with the great need for zinc involved in the normal processing of the prostate gland functions, a lack of which may have caused the molecular injury resulting in the development of prostate cancer (7–11). Low serum levels of zinc have been associated with the increased incidence of prostate cancer (7–12). Previously, Costello et al (8, 9) have shown that *hZIP1* is expressed in PC-3 cells, and that a zinc update actively upregulated by testosterone and prolactin treatment. Furthermore, *hZIP1* expression was regulated by zinc availability. Therefore, when PC-3 cells were exposed to high zinc, *hZIP1* mRNA levels were down-regulated. The molecular mechanisms by which low zinc levels contribute to the development of neoplasia are still obscure, and limited data are available. Costello et al (9) have shown that long-term cellular zinc deficiency leads to an increase in cell proliferation partly because of a reduction in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial apoptogenesis, indicating a physiologic effect of zinc in the regulation of prostate cell growth. Thus, in prostate cancer, 2 themes emerge from the analyses of zinc transporter expression in vivo: (1) the down-regulation of zinc transporters by either genetic inheritance (African descent) or through aging (related to the modulations in the testosterone/prolactin levels or gene expressions acquired with old age) leads to the low accumulation of zinc in the prostate tissues, (12–19) and (2) the loss of the unique capability to retain normal intracellular levels of zinc caused by either the increased export or low import of zinc may be an important factor in the development and progression of malignant prostate cells (1–5,10,29–35). From our data, it appears that the lowest degree of the expression of zinc transporters, *hZIP1* and *hZIP1,* is localized in the areas that exhibit neoplastic lesions, and is less dominant in the areas that are healthy-appearing. Our observation that there are differences in the zinc transport in different racial groups has great significance for prevention. If a role of zinc transporters is clearly established, then a zinc supplementation could be helpful in at least some people. Understanding the molecular events in the pathogenesis of prostate cancer is critical to the evaluation of the natural history of prostate cancer in humans, especially in various racial groups (34–38).

AIM # 3: The Aim of this project was to measure the blood zinc levels in about 2,000 individuals and compare the serum zinc levels in the African-Americans, Africans, Caucasians, Asians, and the mixed race groups. These studies are in progress and we will continue to perform these studies at the Principal Investigator's site-Claflin University-an HBCU. For this purpose, the majority of the serum specimens were to be obtained from the Medical University of South Carolina (MUSC). However, Dr. Tim Garvey moved to University of Alabama at Birmingham and was unable to secure the IRB approval so he can release the sera for the studies. In order to overcome this problem, we made arrangement with a private practice in Virginia. Ettrick Health Canter (20901 Chesterfield Ave., Ettrick, VA 23803: TEL: 804-526-3500) is run by Dr. Loknath Shandilya, MD, PhD, who cares for a large number of patients from both African

American and Caucasians. He has ongoing clinical trials with Pharmaceutical Industries. He will provide the needed samples for our studies. In addition to zinc levels, we will also measure the testosterone and prolactin levels in these groups.

This portion of the study is in progress and the PI has started to collect the well defined specimens from two local clinics in Orangeburg. We have already started to collect the sera. The Claflin Internal Review Board (IRB) has also approved our proposal so we can also collect blood specimens from the students who consent to it. Claflin University has a diverse student population from various racial groups that would provide a rich source of information.

Meanwhile, the PI has already training two students on various methods and they are now carrying out some state of the art techniques to carry out the critical assays at Claflin University. In addition, four more Claflin University Students have signed up to work during the summer to perform some of the critical assays (see below).

Most importantly, we have began to explore the possibility that one of the main reasons that African Americans may have high incidence of diabetes and hypertension is due to lack of appropriate amount of intracellular zinc in the crucial cell types that involves high amount of zinc. The result of our finding are included in the DRAFT of the manuscript which is "in preparation" (please see Appendix II). A summary of our hypothesis is as follows:

"Diabetes Mellitus (DM) is a life threatening disease because of the development of many of the severe secondary complications, including atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormalities, diabetic retinopathy, and ocular disorders. Generally, DM is classified as either an insulin-dependent type I or non-insulin-dependent type II DM. Type I DM is treated only by daily insulin injections; type II DM is treated by several types of synthetic therapeutic substances together with a controlled diet and physical exercise (please see Appendix II).

Type I and type II diabetes are often thought of as diseases with completely distinct etiology. Thus, type I diabetes results from autoimmune destruction of insulin-producing pancreatic islet β-cells, while type II diabetes involves a constellation of metabolic disorders including insulin resistance, impaired control of hepatic glucose production, and β-cells dysfunction. A role for inflammatory mediators in the killing of β-cells in type I diabetes is clearly established. Destruction of β-cells results from direct contact with infiltrating T-cells and macrophages as well as exposure to inflammatory cytokines such as interferon (IFN)-γ, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and reactive oxygen/nitrogen species (ROS/RNS) that such cells produce (). More recently, inflammatory mediators have become increasingly implicated in the development of type II diabetes. Key observations in support of this concept have included demonstration of a role for adipocyte-derived TNFα in the development of insulin resistance as well as studies showing that anti-inflammatory agents such as aspirin or salicylate are able to prevent or reverse insulin resistance via an effect on IκBα and NF-κB activation. Moreover, studies in rodent models of type II diabetes such as the ZDF rat demonstrate an early compensatory increase in β-cell mass as insulin resistance develops, followed by a loss of β-cell mass as insulin secretion declines and frank diabetes ensues. That this loss of β-cell mass might be mediated by inflammatory agents is supported by studies in which lipid-laden islets of ZDF rats, or islets caused to store fat by chronic culture in fatty acids, are much more susceptible to cytokine-mediated cytotoxicity than islets from lean ZDF rats or normal islets cultured in the absence of fat, respectively. It should also be noted that islets are thought to be more susceptible to damage from oxidative stress than other tissues due to their extremely low expression of oxygen radical metabolizing enzymes such as manganese superoxide dismutase (MnSOD), catalase, and glutathione peroxidase. These findings provide motivation for studies on the mechanisms by which inflammatory agents cause functional impairment and cytotoxicity in β-cells, and also spur the development of methods for protecting islet cells against such attack.

The biological relevance of the activation of inflammatory pathways became evident upon the demonstration that interference with these pathways improve or alleviate insulin resistance. The abnormal production of (TNF-α) in obesity is a paradigm for the metabolic significance of this inflammatory response. When TNF-α activity is blocked in obesity, either biochemically or genetically, the result is improved insulin sensitivity. Studies have since focused on the identification of additional inflammatory mediators critical in metabolic control and on understanding the molecular mechanisms by which inflammatory pathways are coupled to metabolic control. Recent years have seen a critical progress in this respect by the identification of several downstream mediators and signaling pathways, which provide the crosstalk between inflammatory and metabolic signaling. These include the discovery of c-Jun N-terminal kinase (JNK) and I $\kappa\beta$ kinase (I kappa K) as critical regulators of insulin action activated by TNF-α and other inflammatory and stress signals, and the identification of potential targets (please see Appendix II)

Zinc and Diabetic African Americans
African Americans disproportionately suffer from various diseases in the US. Many of these diseases include hypertension, lupus, cardiovascular disease, diabetes mellitus, and cancers of the prostate and pancreas. A number of risk factors such as smoking, a high fat diet, little physical activity, stress, and meager access to health care have been the subject of numerous investigations.

In the last half of the 20th century, investigations between the relationships among diseases and micronutrients, such as iron, copper, zinc, and selenium, have been numerous. Of note, the content of zinc in the pancreatic beta cell is among the highest in the body; however, little is known about the uptake and storage of zinc inside this cell. The high zinc requirement of the beta cell relates to each hexameric insulin crystal containing two zinc atoms in insulin granules. When exocytosis of insulin occurs, an insulin granule fuses with the beta cell plasma membrane and releases their contents into the circulation. Thus zinc is secreted along with insulin, thereby requiring the continual replenishment of the beta cell zinc. Because free zinc is toxic to a number of proteins in the beta cell, zinc must be complexed to the specific zinc binding proteins. These transport proteins play a role in zinc uptake and storage in pancreatic beta cells. Interestingly, according to some recent reports, the zinc concentration in patients with Type I diabetes is significantly lower than in healthy controls. These findings are not universal, and other researchers have found no significant differences in the zinc levels between DM and control subjects. However, in these studies racial groups were not studied in a separate manner."

AIM # 4: In a separate but smaller group of individuals, autometallography and atomic absorption spectrophotometry will be used to measure intracellular zinc in various anatomic parts of the prostate tissues.

We have reevaluated our original proposal and we feel that instead of autometallography, Zinc indicators can provide more precise and accurate measure of intracellular zinc in the prostate tissues from the both racial groups. Most important, the autometalography does not allows for the intracellular zinc differences in the malignant verses normal and between the normal prostate tissues from African American and Caucasian. We feel that zinc indicators can provide very important missing information. The following is a brief rational of utilizing zinc indicators and the scientific basis of our reassessment.

Fig 1: Left to Right: i) Zinc uptake measured by Newport Green in the epithelial cells from the peripheral zone (EPZ) of an African American man with PIN. ii) Age match white man with PIN showing significantly higher zinc uptake in the EPZ. iii) Zinc update in a high-grade EPZ of an African American man and iv) in the age-matched white man.

Determination of intracellular zincs concentration by zinc indicators. Total intracellular cellular zinc concentrations ***in situ*** can be measured in the live cells and fresh frozen tissues using the zinc-indicator dyes Newport Green DCF, PDX and TSQ (Molecular Probes, Eugene, OR). As opposed to autometallography, the zinc indicator can provide relative intracellular zinc levels in various anatomical portions of the prostate sections from same individual as well as allows for the comparative analyses of intracellular zinc in the prostates from different racial groups that are age-matched.

Zinc concentrations in the 1–100 nM range can be measured using fluorescent indicators more recently developed indicators with greater Zn^{2+} selectivity (39-49). We have focused our development efforts on probes for detection of higher Zn^{2+} concentrations that are present in prostate peripheral zone (PZ). Peak concentrations of intracellular Zn^{2+} in the PZ of a Caucasian man may exceed 100 μM (unpublished data and please see photos above). We are currently assessing the value of the recently developed FuraZin-1 (F24182, F24183), IndoZin-1 (I24184, I24185), FluoZin-1 (F24180, F24181), FluoZin-2 (F24188, F24189) and RhodZin-1 (R24186, R24187), a series of unique indicators designed for detection of Zn^{2+} in the 0.1–100 μM range with minimal interfering Ca^{2+} sensitivity from Molecular Probes (50-62). The spectral responses of these indicators closely mimic those of the similarly named Ca^{2+} indicators. For instance, FuraZin-1 (57) and IndoZin-1 (44) exhibit Zn^{2+}-dependent excitation and emission spectral shifts, respectively; FluoZin-2 (45) and RhodZin-1 46 (47) show Zn^{2+}-dependent fluorescence without accompanying spectral shifts.

Newport Green DCF and Newport Green PDX Indicators

Since the beginning of 2004 we are evaluating Newport Green DCF indicator (Molecular Probes, N7990, N7991) that has moderate zinc-binding affinity (K_d for Zn^{2+} ~1 µM) but is essentially insensitive to Ca^{2+} (K_d for Ca^{2+} >100 µM), making this a valuable probe for detecting intracellular Zn^{2+} in various portions of the prostate gland. When used alongside dyes with dual Ca^{2+}/Zn^{2+} sensitivity such as fura-2 and mag-fura-2, Newport Green DCF provides confirmation that changes in Zn^{2+} levels, and not Ca^{2+} or Mg^{2+}, are being detected (59-61). Newport Green PDX [48] (N24190, N24191) incorporates the same di-(2-picolyl)amine chelator as Newport Green DCF (Figure 1 above) but has a higher Zn^{2+} dissociation constant (K_d for Zn^{2+} ~30 µM) and a larger Zn^{2+}-free to Zn^{2+}-saturated fluorescence intensity increase. Newport Green DCF has been used to identify insulin-producing β-cells from human pancreatic islets on the basis of their high intracellular Zn^{2+} content (53).

TSQ

Use of the membrane-permeant probe *N*-(6-methoxy-8-quinolyl)-*p*-toluenesulfonamide (TSQ, M688) in cells was first described by Fredrickson (58). TSQ is selective for Zn^{2+} in the presence of physiological concentrations of Ca^{2+} and Mg^{2+} ions (59). The complex of TSQ with free Zn^{2+} apparently has a stoichiometry of two dye molecules per metal atom (62-69), but a 1:1 complex may be formed with metalloproteins. The intracellular Zn^{2+} chelator dithizone blocks TSQ binding of Zn^{2+} (58, 63).

Several reports suggest that TSQ can be used to localize Zn^{2+} pools in the central nervous system (54-56). Zn^{2+} moves from presynaptic nerve terminals into postsynaptic nerve terminals when blood flow is constricted in the brain. This translocation is reported to correlate with ischemia-caused neurodegeneration, as determined by the fluorescence of TSQ (65). TSQ has also been used to detect nitric oxide–induced accumulation of free Zn^{2+} in neuronal perikarya (57) and changes in Zn^{2+} distribution in the rat hippocampus and amygdale (58) during and after kainic acid–induced seizures. TSQ (like Newport

Green DCF) is a selective nontoxic stain for pancreatic islet cells, which have a high content of Zn^{2+}, and may be useful for their flow cytometric isolation (59-62).

TSQ-based assays for Zn^{2+} in seawater and other biological systems exhibit a detection limit of ~0.1 nM (58, 62-65). The simultaneous determination of Zn^{2+} and Cd^{2+} by spectrofluorometry using TSQ in an SDS micelle has also been reported (64). TSQ has been used to measure Zn^{2+} levels in artificial lipid vesicles and live sperm cells by flow cytometry (60).

Recently, we have acquired 60 frozen prostate tissue section from our collaborator at the Medical College of Wisconsin (35 from Caucasian and 25 from African Americans) and are evaluating the intracellular Zn+ levels in these tissues. The initial work has been completed but histological examination and data analyses will take several months to complete. After our analyses we will send these data to our collaborator and he will break the code and determine the accuracy of our initial finding.

AIM # 5: To determine which other factors, including exposure to prolactin, testosterone, external zinc concentrations, the combinations of these three agents and other factors regulate the zinc transporters in the pre-established prostatic cell lines (i.e. PC-3 and La cell lines) and primary cell lines established from the prostatic tumors from various racial groups. We believe that by establishing a link between the low intracellular transport capacity of zinc in the African-American population and the development of prostate cancer, we may be able to design protocols that can increase intracellular zinc levels in the prostate gland. In addition, we hope to identify certain unique genes that may be selectively expressed or suppressed in certain racial groups. These studies may also shed some light on why men from all races develop prostate cancer in old age and how it is linked to intracellular zinc levels and serum zinc, testosterone, and prolactin levels.

The PI has trained two undergraduate students to perform cell cultures on various prostate cell lines. These students have learned the RT-in situ PCR method so they can perform zinc transporter expression assays in vitro. We hope to make significant progress in this Specific Aim in a very short time.

KEY RESEARCH ACCOMPLISHMENTS

- We began with a crucial question: Why are African American men, after 10 to 20 generations in the U.S., twice as likely to develop cancer of the prostate as Caucasian Americans, while South African Blacks are 10 to 30 times less likely to develop prostate cancer than their American distant cousins and 2 to 10 times less likely than African Whites, who may be presumed to have a mixed European ancestry somewhat similar to that of White Americans?

- In order to answer this question in a most definitive fashion, we have divided our possible answers into various categories. The first and foremost of the question was to determine if there are any consistent differences in the expression of significant genes or proteins in the prostate cancers taken from African Americans versus those from White Americans. A study of the genes and proteins which influence the expression of any gene confirmed to be disparately expressed might lead to the identification of one or more environmental or living pattern factor worthy of epidemiological research for its potential relationship to the incidence or progression of prostate cancer in African Americans, Whites, or other groups. For this purpose we chose to evaluate the relative degree of expression of human zinc transporters crucial for retaining the zinc into the prostate.

- We have evaluated 58 prostate cancer tissues in 2 major racial groups (30 from whites and 28 from African Americans) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from White people, the degree of expression of these 2 zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients.

- We also found a significant down-regulation of these two zinc transporters in normal prostate tissues from African American men when compared with age-matched White men.

- We have began to set up the highest state of the art methods to determine the intracellular levels of zinc in the 60 frozen specimens collected until this date.

Our goal is to measure the intracellular zinc levels and the location of zinc inside the various cell types that make up the cancer and normal prostate tissues. Two undergraduate minority students have completed their analyses in the PI's laboratory, and we will continue to critically analyze the patients' tissue specimens in the upcoming months.

- We already have trained two additional undergraduate minority students to carry out tissue culture methods on well-defined prostate cancer cell lines. These cell lines will be analyzed for the relative expression of zinc transporters before and after the exposure to various concentrations of zinc, testosterone, and prolactin. We hope to complete these studies by the end of our fiscal year.

- The blood samples that were supposed to come from MUSC will not be available due to the relocation of our collaborator to UAB. We have initiated collaborations with two local clinics in Orangeburg as well at out own institute to collect the specimens. In addition, we have developed a formal collaboration with a clinical group in Virginia, which will provide the blood specimens we need to carry out our studies. We will be able to do so and would be able to collect over 2,000 blood specimens from all major races to evaluate concentrations of zinc, testosterone, and prolactin in African Americans versus Whites.

- We are certain that there is a direct link between zinc transport and prostate cancer. If a strong link is established between the environment, genes, and diet, then a special nutritional formula, medication, or other intervention might be especially designed to test the ability to decrease the incidence of this disease in African Americans. Such an intervention, if successful, might be useful for the persons of all populations.

REPORTABLE OUTCOMES:

Manuscript (published)

A manuscript was published and attached as Appendix 1. This article appeared in a peer-reviewed journal in Sept 2003.

Rishi I, Baidouri H, Abbasi JA, Bullard-Dillard R, Kajdacsy-Balla A, Pestaner JP, Skacel M, Tubbs R, Bagasra O. Prostate cancer in African American men is associated with downregulation of zinc transporters. Appl Immunohistochem Mol Morphol. 2003 Sep;11(3):253-60. **(Appendix I)**

Manuscripts (Submitted)

1. Mohammed I. Alhatou, John T. Sladky, Omar Bagasra, and Jonathan D. Glass. 2004· Mitochondrial Abnormalities in Dermatomyositis: Characteristic Pattern of Neuropathology. (In the Press: **Histochem J**).

2. Omar Bagasra, and Kiley R. Prilliman. 2004. RNA Interference: Tip of the Iceberg. (In the Press: **Histochem J**).

3. Bagasra, O. Bobroski, L., Alexander U Bagasra, Deepa Patel, P. Saikumari, Aura R. Davidson, El- Roiey, and Charles Wood. 2004. Presence of Human Herpes Virus Type 8 in Human Semen by in situ PCR. (Submitted for Publication: **J. Infectious Diseases).**

4. Bagasra, O, Harold W. Lischner, Lucia Pirisi-Creek, Kim Creek, Alexander U. Bagasra, and Jeremy S. Lee· 2004. Immunity to Lentiviruses Requires Endogenous Small RNAs that Form an Intramolecular Triplex Nucleic Acid Structures with Preintegration Complexes (To be submitted to ***Cell).***

Manuscripts (In Preparation).

Irum Rishi, Sibrina Collins, and Omar Bagasra, MD, PhD. Role of Zinc in Diabetic African Americans **(Appendix II).**

Bagasra, O, R Bullard-Dillard, and J P Pestaner (2004). The Survival of Viviparous Mammals May Be Due to Expression of Endogenous Retroviruses during Gestation (To be submitted: **J. Reproductive Immunology**).

Abstracts: The following Abstract has resulted from this award:

Student Presentations and Abstracts:
Anita Carter & Omar Bagasra. Role of Zinc Indicators in the Intracellular Determination of Zn+ in Normal and Malignant Portions of Prostate Gland. SC Life Colloquium of Undergraduate Research. April 3, 2004.

Presentation: The PI presented data on prostate cancer at the following locations:

- Bagasra, O. New Frontiers in Morphology. 10th International Conference of molecular morphology. Oct 5-8, 2002. Santa Fe, NM.
- Bagasra, O. A New Idea in Prostate Cancer Prevention. Invited speaker at San Francisco State University. San Franciasco, CA. May 22, 2003.
- Bagasra O. RNAi: a new revolution in molecular biology. Annual Meeting of the Am. Society of Investigative Pathology. Session 275. Trends in Experimental Pathology: Frontiers in Molecular Morphology Translational Research. Wash. D.C., April 18^{th} 2004. FASEB Pg 103-104. April 17-21, 2004.

Patents and licenses applied for and/or issued: A patent is being prepared on the effects of zinc supplement in the prevention of Prostate Cancer in the African American Population.

Degrees obtained that are supported by this award:

Two minority students worked on this project as part of the requirement of their undergraduate degree:

- Tiffany Brown
- Melodie Harrison
- Anita Carter

Development of cell lines, tissue or serum repositories; infomatics such as databases and animal models, etc. None

Funding applied for based on work supported by this award:

An application was submitted to NCI based on the preliminary result from this award and would be funded. The details are as follows;

FUNDED BY the National Cancer Institute

NCI: 08/01/2003 to 04/31/06 "Training grant for Claflin University Students"
In collaboration with USC Cancer Center
$566,035/yr with USC Cancer Center, P.I.: O. Bagasra

PENDING at DoD

DOD PC040560 10-1-2004 to 9-31-2007 $566,000 for 3 yrs.
Role in the Project: PI
"MOLECULAR TARGETS FOR ZINC IN PROSTATE CANCER PREVENTION"

Employment or research opportunities applied for and/or received based on experience/training supported by this award. YES!

CONCLUSIONS

We started our project to address a very important question concerning why African American men, after 10 to 20 generations in the U.S., twice as likely to develop cancer of the prostate as Caucasian Americans, while South African Blacks are 10 to 30 times less likely to develop prostatic cancer than their American distant cousins and 2 to 10 times less likely than African Whites, who may be presumed to have a mixed European ancestry somewhat similar to that of White Americans. Are there any consistent differences in the expression of significant genes or proteins in the prostate cancers taken from African Americans versus those of White Americans? A study of the genes and proteins which influence the expression of any gene confirmed to be disparately expressed might lead to the identification of one or more environmental or living pattern factors worthy of epidemiological research for its potential relationship to the incidence or progression of prostate cancer in American Blacks, Whites, or other groups.

We have concluded that there are significant differences between Whites and African Americans with regards to the degree of expression of two zinc transporters that are involved in importing zinc from the outside into the prostate glands. There are many additional assays that need to be performed. We feel that intracellular zinc levels by Zn+ indicators would provide a strong support for this hypotheses, if Zn+ levels in the malignant Verses Normal and African American Verses Caucasians support our initial report. Once these data are confirmed in larger groups, this finding could have a significant application as a preventive maneuver at least in African Americans. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

Also, African Americans disproportionately suffer from various diseases in the US. Many of these diseases include hypertension, lupus, cardiovascular disease, diabetes mellitus, and cancers of the prostate and pancreas. A number of risk factors such as smoking, a high fat diet, little physical activity, stress, and meager access to health care have been the subject of numerous investigations. However, the factor of the interaction between genetics and the environment has received very little attention in the basic scientific community. This work is being carried our currently in the PI's laboratory and a manuscript is included as Appendix II for the review.

According to numerous epidemiological data in the 21st century, patients suffering from DM will increase more than in the 20th century. For those reasons, the creation and development of a new class of pharmaceuticals for the treatment of DM in the 21st century is extremely desirable. In the last half of the 20th century, investigations between the relationships among diseases and micronutrients, such as iron, copper, zinc, and selenium, have been numerous.

Our laboratory is investigating the potential role of zinc transporters in the pathogenesis of many illnesses that disproportionately affect the African American community.

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Prostate Cancer in African American Men Is Associated With Downregulation of Zinc Transporters

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In the United States, prostate cancer is the most commonly diagnosed male cancer and the second leading cause of all male cancer deaths. Furthermore, incidence rates are higher in African Americans than in any other racial group. Our laboratory is attempting to decipher the environmental and molecular mechanisms involved in the development of prostate cancer in African Americans. Because Africa is a mineral-rich continent, and the zinc levels in the water and diet are high, it is hypothesized that Africans may have genetically downregulated their zinc absorption capacity; otherwise, they would absorb abnormally high levels of zinc, resulting in various serious neurodegenerative and biochemical disorders. It is therefore possible that people of African origin may have a lower capacity to absorb zinc when compared with other racial groups because of their inherent downregulation of zinc transporters. Extensive research has shown that low serum levels of zinc are associated with the increased incidence of prostate cancer. We have evaluated 58 prostate cancer tissues in 2 major racial groups (30 from whites and 28 from African Americans) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from white people, the degree of expression of these 2 zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients. We also found a significant downregulation of these 2 zinc transporters in normal prostate tissues from African American men when compared with age-matched white men. The loss of the unique ability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. Our observation that the uptake of zinc may be different in racial groups is intriguing and relevant. Once these data are confirmed in larger groups, this finding could have significant application as a preventive maneuver for at least for some people. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk.

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The prostate contains high amounts of free zinc ions that are excreted into the seminal fluid. The extracellular and intracellular distribution of zinc ions in the rodent using highly specific autometallographical studies have shown that zinc accumulates primarily in the acinic lumen of the lateral lobes, whereas the dorsal lobe stains only faintly and the ventral lobe is void of grains.[1,2] At the ultrastructural levels, the presence of zinc ions is confined to apical secretory vesicles and the epithelium of mainly the lateral lobes in both rodents and humans.[1,3] Recently, Iguchi et al,[4] using semiquantitative reverse-transcriptase polymerase chain reactions (SQ-RT-PCRs), showed that the expression of zinc transporter (ZnT) 2 in rats was very high in the lateral and dorsal prostate and much lower in the ventral prostate. In humans, it appears that zinc ions are constantly secreted from the epithelial cells into both the acinic lumen and the intercellular canaliculi.[5] Prostate secretory epithelial cells have the unique function and capability of accumulating extremely high intracellular levels of zinc.[3–5] One of the effects of this accumulation is the inhibition of cell growth, partly because of an increase in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial apoptogenesis.[6] Prolactin and testosterone regulate zinc accumulation in the prostate; however, little information is available concerning the mechanisms associated with zinc accumulation and its regulation in prostate epithelial cells.[7,8] By using the human malignant prostate cell lines LNCaP and PC-3, Costello et al[7] have shown that the zinc accumulation in both cell types is stimulated by physiologic concentrations of prolactin and testosterone. Their studies reveal that these cells possess the ability to uptake zinc rapidly, indicative of the presence of a plasma membrane high-affinity zinc transporter, possibly by the regulation of the ZIP-type zinc transporter gene expression.[7,8] Kinetic studies demonstrate that the rapid uptake of zinc is effective under physiologic conditions that reflect the total and mobile zinc levels in circulation.[8] Correspondingly, genetic studies demonstrate the expression of a ZIP family zinc uptake transporter in both LNCaP and PC-3 cells.[8] Some of these zinc-accumulating characteristics are found to be specific for prostate cells. These studies support the concept that prostate cells express a unique hormone-responsive, plasma membrane-associated, rapid zinc uptake transporter gene that is associated with their unique ability to accumulate high zinc levels.[9–11]

In the United States, the incidence of prostate cancer is significantly higher in African Americans than in white people or Asian Americans.[12–19] We hypothesized that because Africa is a mineral-rich continent and zinc levels are relatively high in the water and diet, abnormally high amounts of zinc in the blood may result in various neurologic and metabolic abnormalities. The zinc absorption and transport systems are genetically downregulated in the African populations. The phenomenon may be similar to sickle cell anemia, in which a single mutation has provided the survival advantages against the ravages of the fatal form of malaria.[20] We hypothesize that when African people entered North America, mostly during the slave trade, they encountered an environmental problem: in North America, the zinc levels are relatively low, and the native populations (the American Indians) and other races who migrated from Europe and Asia have a higher capacity to transport zinc to various organs, especially to the prostate gland, in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in the white and black populations.[21] Here the situation is reversed. The white population is unable to upregulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect them from damaging solar ultraviolet (UV) light. Squamous cell carcinoma is the most common tumor of sun-exposed epithelium in white populations.[21] Therefore, just as white people carry an evolutionary disadvantage against the solar UV rays outside of their ancestral low-UV light environment, the low absorption capacity of zinc has created a disadvantage in the peoples of African descent who have migrated outside Africa. If this is the case, long-term low serum concentrations of zinc deprive the prostate gland of its essential source of vital trace mineral ingredients, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the idea that low levels of zinc contribute to the high incidence of prostate cancer.[1–10]

In this research study, it was our goal to test this hypothesis by analyzing the prostate tissues for their ability to express 2 major zinc transporters responsible for accumulation of zinc in the prostate glands. For this purpose, we used a highly sensitive RT-in situ-PCR method to compare the relative levels of expression of the 2 zinc transporters, *hZIP1* and *hZIP2*, in 2 racial groups in the United States (white and African American).

METHODS AND MATERIALS

Human Subjects and Study Protocol

Archival, formalin-fixed, paraffin-embedded specimens of primary prostate carcinoma were retrieved from the files at the Department of Pathology of the Medical College of Wisconsin. Similarly, fixed tissues from radical prostatectomy specimens were obtained from the Cleveland Clinic Foundation, according to the approved protocols of the respective institutes. Normal prostate tissues were autopsy specimens obtained from the State of Maryland Medical Examiner's Office at Baltimore, Maryland.

Quantitative Reverse-Transcriptase Polymerase Chain Reaction Zinc Transporters

The total mRNAs were harvested from the deparaffinized tissues as described previously.[22–24] The RNA preparations were used to quantitate the levels of zinc transporters, and the relative levels of expression were visualized according to each racial subgroup.

Semiquantitative Reverse-Transcriptase Polymerase Chain Reaction

The details of the SQ-RT-PCR have been described previously.[22–24] The major advantage of this protocol that allows the relative quantitation of each of the specific RNA species in the samples is the use of standard curves of in vitro transcribed mRNAs of *hZIP2*. Plasmids containing full-length *hZIP2* and pCMV-hZIP2 were kindly provided by Dr. David J. Eide of the Department of Nutritional Sciences, University of Missouri. This clone was used to develop a standard curve to semiquantitate the relative degree of the expression of *hZIP2*. The full-length *hZIP2* shows a significant homology to the members of the *ZIP* family, including *hZIP1*. Therefore, we were able to design primer pairs that could amplify the conserved sequences of hZIP1 and hZIP2 by multiplex polymerase chain reaction. The concentrations of the mRNAs were measured spectrophotometrically, and the relative copy numbers of mRNAs present in 1 μL of solution were calculated using the molecular weight of the transcript and the Avogadro number. Relative numbers of each *ZIP* were derived by SQ-RT-PCR and generated by a serial 2-fold dilution of *pCMV-hZIP2* plasmid DNA. In the linear amplification range of these curves, the copies of in vitro transcribed mRNAs were plotted against the relative size of the amplified bands of the amplified fragments of the 2 *hZIPs*. Using these dilution curves of the plasmid (performed in duplicate), the relative number of the spliced mRNAs for *hZIP1* and *hZIP2* were calculated. All samples were tested in at least 3 independent experiments. As a control, we performed quantitative RT-PCR of β-actin, as we described previously.[22–24]

Reverse-Transcriptase In Situ Polymerase Chain Reaction

Paraffin sections from 58 prostate biopsies of men with clinical histories of prostate cancer, and 4 from autopsy specimens of people with normal glands who died of automobile accidents, were processed for RT-in situ-PCR. Briefly, paraffin-embedded tissue sections of the specimens were received from each of our collaborators in a blinded fashion. All the reagents were prepared in RNase-free reagents. Therefore, all the slides were deparaffinized with sequential washings with EM-grade xylene, absolute alcohol, 95% ethanol, and 70% ethanol for 5 minutes each, then washed twice in a 1× phosphate-buffered saline (PBS). After deparaffinization, these slides were further fixed in a Streck fixative (STF; Streck Labs, Inc, Omaha, NE) for 2 hours. Incubating slides in 3× PBS for 10 minutes inactivated STF. The slides were washed twice in 1× PBS. These slides were treated with proteinase K (6 μg/mL) at room temperature for 22 minutes. Proteinase K was inactivated by incubating slides on a heat block at 95°C for 5 minutes. To perform the amplification of mRNA sequences for *hZIP1 and hZIP2*, we used multiply spliced sequences that flank the junctions of 2 exon splice sites. Because these RNA-specific primers will not amplify the genomic DNA template, one can perform the amplification of multiple mRNAs simultaneously. The following primer pairs were used: sense 5′-ACCAGACAAGGAC-TTCA-ATTAC-3′ and antisense 5′- GAGGACTAAAGCTGA-AAACATC-3′ for *hZIP1*, and sense 5′-GAATCACAG-ATTCAGAAGTTCA-3′ and antisense 5′-CTCTCCAT-AGGGATACTC CATA-3′ for *hZIP2*. The amplification of β-actin mRNA was performed by using a pair of primers: 5′-ATCTGGCACCTTCTACAATGAGCTGCCG-3 and 5′-CGTCATACTCCTGATTGCTGATCCACA-CATCTGC-3′. The *hZIP2* gave a 102-bp product and *hZIP1* gave a 189-bp product. The β-actin pair yielded 838-bp amplicons.[22–24] To amplify, we used the *rTth* enzyme, which has both the RT and polymerase function.[23] The amplification cocktail contained the pair of primers at 100 pM each in 50 mM Tris pH 8.3, 8.5 mM $MgCl_2$, 10 mM $MnCl_2$, 40 mM KCL, 1 mM dithiothreitol, 10× transcription buffer, 10× chelating buffer, 5 U *rTth* recombinant thermostable DNA polymerase) enzyme, and 200 mM of each deoxyribonucleoside triphosphate. Twenty μL RT (reverse transcriptase enzyme) cocktail was added to each slide. The slides were then sealed with slide frame sealer and inserted into the slide slots of a thermocycler specially designed for in situ PCR (MJR-Twin Tower, PTC 200, Waltham, MA). The 2 cycles were programed for 30 minutes at 62°C, the 94°C for 2 minutes (for cDNA step), and then cDNAs were amplified for 30 cycles at 92°C denaturing, 55°C anneaing, 72°C extension.

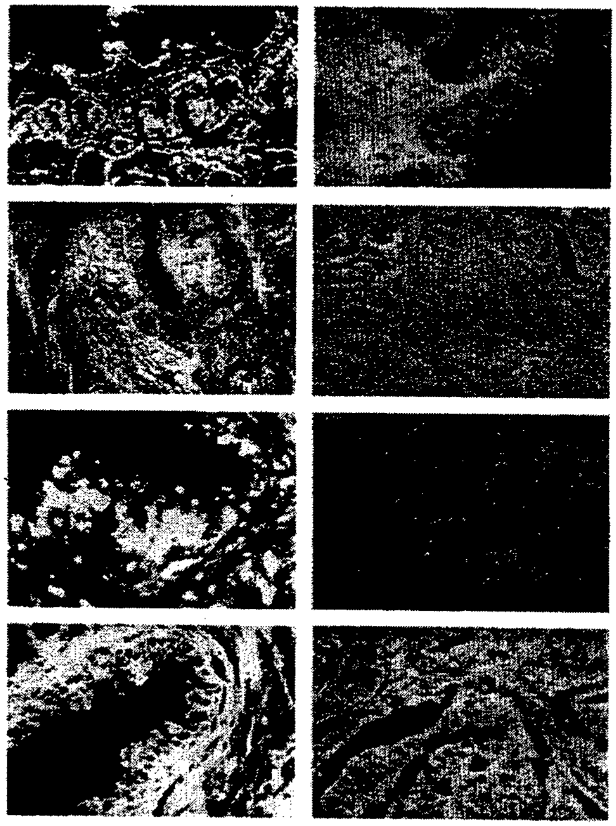
Hybridizations were performed with either Cy3 = 2-amino-3-mercaptopropionamide or an FITC (fluorescen isothiocynate) oligonucleotide probe for the *hZIP1* and *hZIP2* sequences (oligonucleotide: 5′-CAGC-AAGTGAGAGAAATTCTTCTGGTGATGCTGATTC-AGCTC-3′ and 5′-CTTAGAATTTCAGTGGAGTC-TTTTTCCTCTTGCAGTTTAAAGCAAAAGTC-3′). Hybridizations were performed in a buffer containing 50% formaldehyde, 10 mM dithiothreitol, 2× sodium chloride/sodium citrate solution, 100 μg/mL fragmented salmon sperm DNA, 2% bovine serum albumin, 1 mg/mL *Escherichia coli* tRNA, and 20 pmol probes at 95°C for 2 minutes, then 40°C for 18 hours. These tissue sections were then washed to remove unbound probes and viewed under UV epifluorescence microscopy after the cells were washed. To preserve the intensity of the hybridized probes, the tissues were not counter-stained. Parallel hematoxylin and eosin-stained slides were used to identify various histologic cell types in the tissue sections. Microscopic examination usually reveals cytoplasmic staining for mRNA versus nuclear staining for DNA.[22–24] Cell enumeration was performed on coded slides by at least 2 pathologists.

RESULTS

Degree of hZIP1 and hZIP2 Expression in the Malignant Prostate Tissues From White and African American Men

We evaluated the *hZIP1* and *hZIP2* expression, the 2 major zinc transporters, by simultaneously performing a multiplex RT-*in situ*-PCR. We evaluated 58 prostate cancer specimens in a blinded manner for their level of expression of these 2 zinc transporters. The majority of the specimens were from patients who had a 3+3 or 3+4 Gleason score. Upon unlocking the blinded codes, all the specimens from the white men exhibited a significantly higher degree of expression of the 2 zinc transporters than the majority of the specimens from the African Americans. Therefore, all 30 specimens from the white men's prostate biopsies exhibited a modest degree of expression of both of the zinc transporters, whereas 26 of 28 prostate specimens from the African Americans exhibited a low or very low expression of both the zinc transporters. In 1 of the other 2 specimens from African Americans, the prostate sections exhibited high expression of *hZIP1* and low expression of *hZIP2*, whereas in the second case, a reverse pattern of expression was observed. In Figure 1, we have shown representative microphotographs of 8 prostate intraepithelial lesions from age-matched specimens, 4 from each racial group.

Figure 2 shows high-grade prostate carcinomas from 6 age-matched specimens, 3 from each racial group. As it is evident in Figures 1 and 2, the degree of expression of both zinc transporters is significantly higher in the prostate tissues from white men than in the age-matched



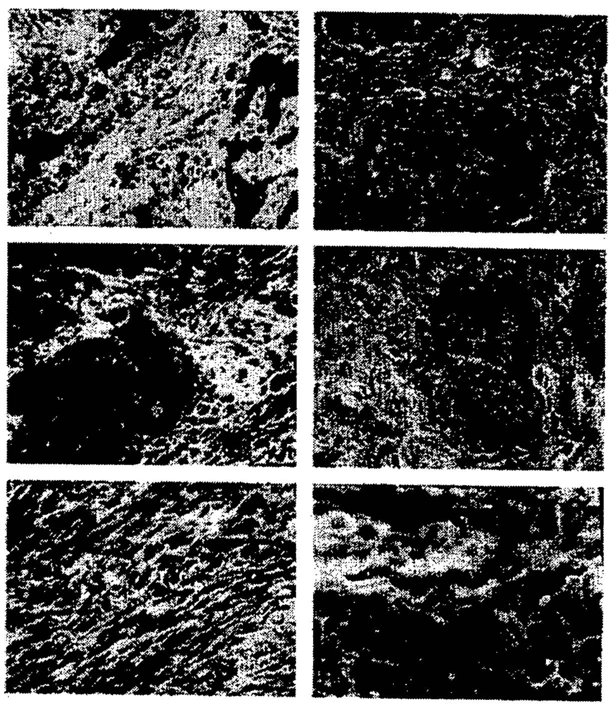
Caucasian men African American men
Green = *hZIP2*
Red = *hZIP1*

FIGURE 1. Representative photomicrographs showing the expression of 2 zinc transporters in intraepithelial lesions, *hZIP1* and *hZIP2*, by multiplex RT-in situ-PCR. Four intraepithelial neoplastic lesions from white men (left) and age-matched specimens from African American men (right) are shown. The coexpression of the zinc transporters, *hZIP1* (red) and *hZIP2* (green), the neoplastic lesions and areas surrounding the tumor lesions exhibit a wide variation in the coexpression of the zinc transporters, *hZIP1* (red) and hZIP2 (green), in the white group. In African Americans, the prostate cancer from the tumors and surrounding areas exhibited a markedly decreased expression of the zinc transporters compared with the tumors from the white patients.

lesions from the African Americans. The coexpression of the zinc transporters *hZIP1* and *hZIP2* in the neoplastic lesions and areas surrounding the tumor lesion showed a wide variation in the expression of these zinc transporters in all tissues from both races. However, with the exception of 2 cases, we observed a consistent decreased degree of the expression in prostate tissues from African Americans over that seen in their counterparts, regardless of their tumor grade. The prostate cancer from the African Americans' tumors and surrounding areas exhibited a markedly decreased expression of zinc transporters compared with the tumors from the white patients. Of note, in all cases, the expressions of both the zinc transporters were visibly lower in the neoplastic areas compared with the surrounding normal-appearing areas. This finding is consistent with the data indicating that overall, the zinc levels are lower in the malignant portions of the prostate gland.[3]

We also performed SQ-RT-PCR analyses in the mRNAs isolated from the prostate tissues of the 8 men shown in Figure 1 for *hZIP1*, and also from 6 people for hZIP2, shown in Figure 2. As shown in Figure 3, the relative expression in the specimens from African Americans of both zinc transporters, *hZIP1 and hZIP2*, were significantly lower when compared with their age-matched counterparts.

To demonstrate that mRNAs were not degraded in the paraffinized prostate specimens, we analyzed the presence and integrity of β-actin mRNAs by RT-PCR. RT-PCR for β-actin was performed on all the specimens. As shown in Figure 3, 8 specimens evaluated for *hZIP1* also had intact mRNAs for β-actin, which clearly demonstrate the integrity of mRNAs in the specimens we analyzed for the ZIP transporters. More importantly, there was no significant difference in the degree of amplification between the specimens isolated from white men and



Caucasian men African American men
Green = *hZIP2*
Red = *hZIP1*

FIGURE 2. Representative photomicrographs showing the expression of the 2 zinc transporters in high-grade tumors, *hZIP1* and *hZIP2*, by multiplex RT-in situ-PCR. Shown are 6 intraepithelial neoplastic lesions, age-matched specimens, 3 from each racial group: white (left) and African American men (right). In African Americans, the prostate cancer from the tumors and surrounding areas exhibited a marked decrease in the expression of the zinc transporters compared with the tumors from the white patients.

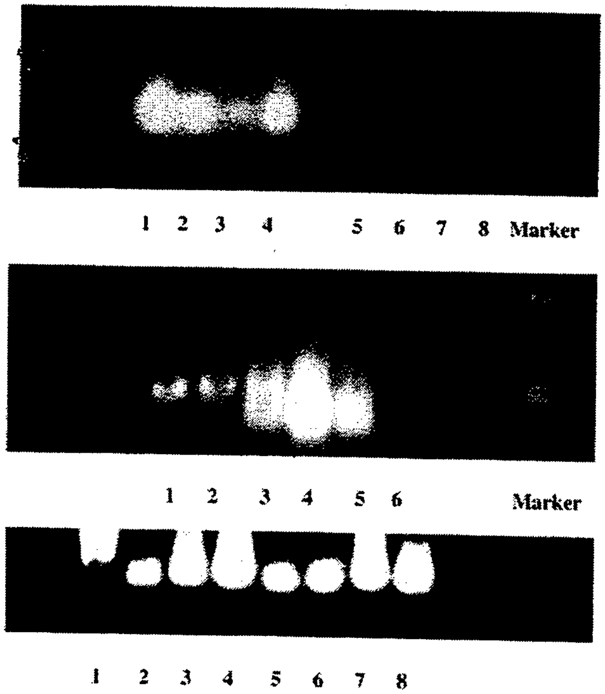


FIGURE 3. Semiquantitation of spliced mRNAs of *hZIP1* and *hZIP2* zinc transporters by QS-RT-PCR. (Top) mRNAs from each specimen were isolated from the 8 people shown in Figure 1. RT-PCR was performed. Ethidium bromide-stained gel electrophoresis of *ZIP1* shows the relative degree of the amplifications. Lanes 1, 2, 3, and 4 are from white tissues, whereas lanes 5, 6, 7,and 8 are from the specimens from African Americans. (Middle) Amplifications of *hZIP2* from the total cellular mRNA isolated from the 6 neoplastic lesions. These specimens were randomized, and RT-PCR was performed for *hZIP2* on the 6 specimens shown in Figure 2. Lanes 1, 2, and 5 are from specimens from African Americans. Lanes 3, 4, and 5 are from specimens from white subjects. (Bottom) mRNAs isolated from the 8 specimens and tested for hZIP1 (top) were also tested for the presence and integrity of β-actin mRNA. Amplification of β-actin from the total cellular mRNA was successful, and there was no significant difference in the degree of amplification between the specimens isolated from white people and African Americans.

African Americans. These analyses validated 2 important points: (1) the mRNAs we isolated were intact, and (2) the differences we observed in the expressions of the zinc transporters were not caused by relative degradation of mRNA signals in different specimens.

In 2 of 28 specimens from African Americans, we observed a significant upregulation of 1 of the 2 *hZIP* transporters, but not both. As shown in Figure 4, two prostate neoplastic lesions exhibited an overexpression of either *hZIP1 or hZIP2*, but not both. The inheritance of the overexpression of *hZIP1 or hZIP2* could have resulted from the interbreeding that occurred in past generations between the white and African American parents (or grandparents) of these people. This could have resulted in the correction or overcorrection of the genetic downregulation of the *hZIP1* or *hZIP2* transporters. These observations also point toward a promoter-mediated regulation of these 2 zinc transporters. This possibility is currently being evaluated in our laboratory.

To determine whether the relatively low expression of *hZIP1 and hZIP2* in African Americans is limited only to neoplastic areas, we examined the prostate tissues from normal, nonneoplastic tissues from healthy men of both races who died because of automobile accidents. As shown in Figure 5, there is a high degree of the expression of both *hZIP1* and *hZIP2* within the normal prostate tissues from 3 normal white males, whereas the expression in the prostates of 2 healthy African Americans was, at best, moderate.

DISCUSSION

African Americans have the highest prostate cancer incidence rate in the world.[13–18,25] At a global level, the rates of incidence are low in Asian and African men, low to moderate in white men, and high in African American men.[13,25] Using data collected between 1988 and 1992, Wingo et al[14] reported that African Americans have a 35% higher incidence rate and a 223% higher mortality

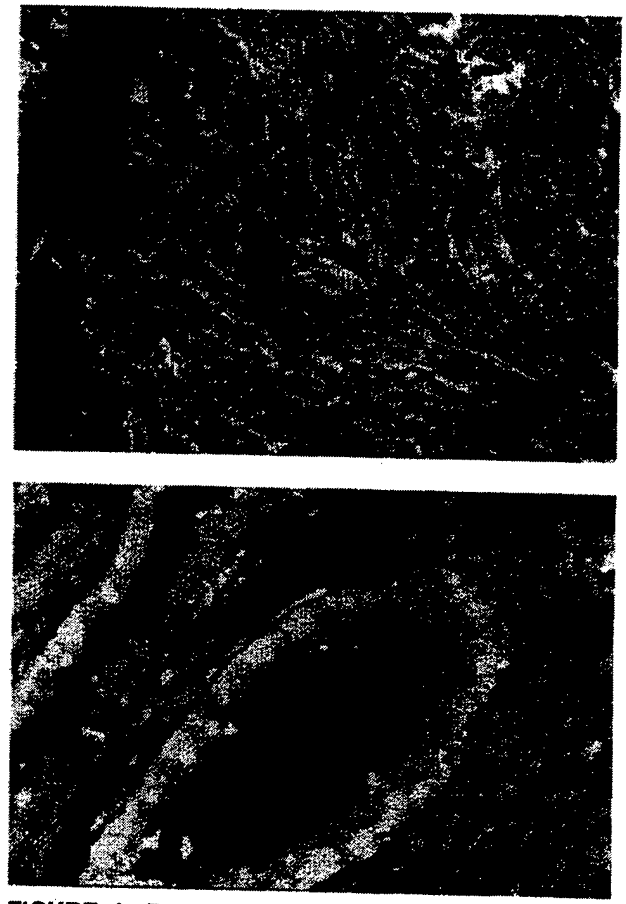


FIGURE 4. Representative photomicrographs from 2 neoplastic specimens from African American men. (Top) Upregulation of *hZIP1* (red) and relative downregulation of *hZIP2*. (Bottom) Upregulation of *hZIP2*, whereas *hZIP1* expression is almost absent.

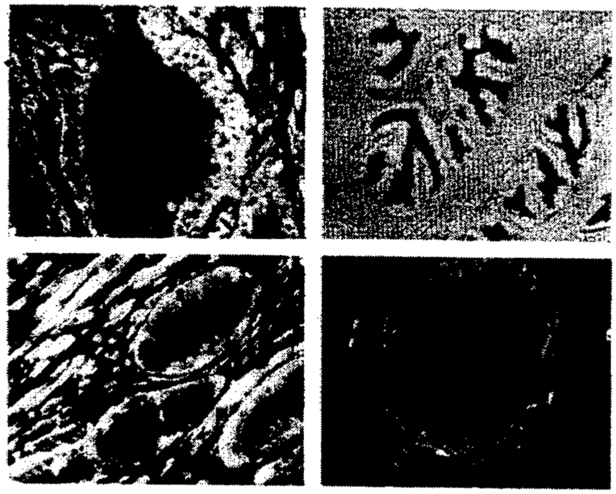


FIGURE 5. Representative photomicrographs showing the relative expression of 2 zinc transporters, *hZIP1* and *hZIP2*, in the normal prostate glands by multiplex RT-in situ-PCR. The relative expression of *hZIP1 and hZIP2* in 2 normal prostate tissues from white men (left) and 2 age-matched African American men. Note that there is a high degree of expression of both *hZIP1* and *hZIP2* within the normal prostate tissues from 2 normal white men, whereas the expression in the prostates of 2 normal African American men would be at best scored as moderate.

rate from prostate cancer when compared with whites. The differences in the incidence and mortality between African Americans and whites are attributed to screening, environmental, and biologic factors.[16,17] When compared with white controls, black men present at a younger age with a higher grade and stage of the disease for their age, and with a greater delay in diagnosis.[18,19] Whether the pathogenesis of prostate cancer is different in African American men compared with white men remains unanswered. Whittemore et al[18,36] note that African American men appear to have a larger volume of "latent prostate cancer." These investigators believe that larger-volume latent carcinomas are those that progress to become clinically evident at a faster rate, suggesting that the events that account for racial differences in prostate cancer incidences may occur very early in cell transformation, and thus may be genetically controlled.

We have hypothesized that the major reason for the high incidence of prostate cancer in African Americans may be their inherent inability to absorb or transport normal amounts of zinc from the Northern American environment, in which there is a relatively low concentration of zinc in the diet. We have further hypothesized that zinc absorption and transport systems are genetically downregulated in some of the African populations. Such natural selection may occur because of the serious adverse effects on the nervous system caused by high zinc levels.[26–28] However, when African people entered North America, mostly during the slave trade, they may have encountered an environmental disadvantage because of their inherent downregulation of zinc transporters. On this continent, the zinc levels are relatively low, and the native populations (the American Indians) and other races who migrated from low zinc areas, ie, Europe and Asia, have a higher capacity to absorb zinc and are able to transport it to the other organs in an appropriate manner. This genetic regulation can be compared with the expression of melanin pigments in white and nonwhite populations. The white population is unable to upregulate their melanin pigmentation sufficiently, even in the presence of strong sunlight, to protect themselves from the damaging solar UV light of wavelengths between 290 and 320 nm. Skin cancer is the most common type of cancer in the United States: more than 600,000 cases of skin cancer are reported each year in this country in the white population. Squamous and basal cell carcinomas are the most common tumors of sun-exposed skin areas in this group.[21] Just as white people carry an evolutionary disadvantage against the solar UV light outside their low UV light ancestral environment, the low absorption capacity of zinc has created a disadvantage in people of African descent when they migrate outside Africa. Long-term low serum concentration of zinc deprives the prostate gland of its essential source of a vital trace mineral ingredient, resulting in prostate metaplasia and neoplasia. A large body of the scientific literature and careful studies support the association of low levels of zinc with prostate neoplasia.[3–6] Zinc is an essential nutrient to all organisms because it is a required catalytic or structural cofactor for 100s of zinc-dependent enzymes and other proteins such as transcription factors. An example of effect of low serum levels of zinc can be seen in the animal model of *lethal milk* mouse mutant.[29] ZnT4 is deficient in the *lethal milk* mouse mutant, in which pups of any genotype suckled on homozygous *lethal milk* mothers die of zinc deficiency before weaning. The zinc level in the milk of homozygous *lethal milk* animals is approximately 50% that of normal animals, demonstrating that ZnT4 plays a crucial role in development.[29] Various reports suggest that regardless of race and geographic location, the etiology of certain prostate carcinomas may be linked to zinc transporters. Because the expression of zinc transporters also appears to be regulated by prolactin and testosterone, an age-related increase in the incidence rate of this malignancy may also be indirectly linked to the zinc uptake by the prostate gland.[8–11]

Members of the *ZIP* family are found in all cellular life forms, including archaebacteria, eubacteria, and eukaryotes.[10,30–34] There are currently approximately 85 members reported in the sequence databases. These fall into 4 subfamilies based on their amino acid similarities.[10] Most members are predicted to have 8 transmembrane domains and share a predicted topology where the amino and carboxyl termini are extracytoplasmic. There are 12 known ZIP members in the human genome.[31] Three of the human proteins, *hZIP1*, *hZIP2*, and *hZIP3*, are very closely related to the fungal and plant proteins known to be zinc uptake transporters. *hZIP2* expression has been detected only in prostate[31] and uterine[32] epithelial cells, suggesting that this protein plays a very specialized tissue-specific function. On the other hand, hZIP1 is expressed in all 24 human tissues examined so far.[33] Gaither and Eide[10,11,31] have sequenced and characterized the *hZIP2* gene, a human zinc transporter identified by its similarity to zinc transporters recently characterized in fungi and plants. *hZIP2* is a member of the *ZIP* family of eukaryotic metal ion transporters that includes 2 other human genes, *hZIP1* and *hZIP3*, and the genes in mice and rats.[10,11]

The human genome contains at least 3 *ZIP* family members. The current hypothesis is that these genes encode zinc uptake transporters.[10,11] We can gain some insight into *ZIP* function in humans by considering the tissues in which these proteins are expressed. Repeated attempts by Gaither and Eide[11] to detect *hZIP2* mRNA on Northern blots of poly (A)+ RNAs derived from different human tissues and cultured cell lines failed to produce positive results. It appears that the *hZIP2* transporter gene is normally expressed at low levels and in specific cell types, and that a more sensitive detection method is required. We also attempted to quantitate *hZIP2* mRNA by Northern blots; however, several attempts were not productive. Therefore, we decided to use highly sensitive RT-in situ-PCR and SQ-RT-PCR methods. Gaither and Eide[10,11] isolated only 4 *hZIP2*-expressed sequence tag clones found only in prostate and uterine cDNA libraries. The observation that these particular tissues express *hZIP2* may be instructive in that cells of the prostate contain the highest zinc level of any soft tissue in the body. Any potential downregulation in this transporter may play a pivotal role in the pathogenesis of prostate cancer. Thus, it appears that the expression of *hZIP2* in prostate and uterine tissues may help meet their particular needs of zinc metabolism. In contrast, the low-affinity *hZIP1* and *hZIP3* have been cloned as expressed sequence tags from a large number of different tissues, indicating that these genes are widely expressed and may play general housekeeping roles.[10]

Therefore, observed zinc transporter expression may be associated with the great need for zinc involved in the normal processing of the prostate gland functions, a lack of which may have caused the molecular injury resulting in the development of prostate cancer.[7–11] Low serum levels of zinc have been associated with the increased incidence of prostate cancer.[7–12] Previously, Costello et al[8,9] have shown that *hZIP1* is expressed in PC-3 cells, and that a zinc uptake was actively upregulated testosterone and prolactin treatment. Furthermore, *hZIP1* expression was regulated by zinc availability. Therefore, when PC-3 cells were exposed to high zinc, *hZIP1* mRNA levels were downregulated. The molecular mechanisms by which low zinc levels contribute to the development of neoplasia are still obscure, and limited data are available. Costello et al[9] have shown that long-term cellular zinc deficiency leads to an increase in cell proliferation partly because of a reduction in apoptosis. The accumulation of high intracellular levels of zinc by prostate cells induces mitochondrial apoptogenesis, indicating a physiologic effect of zinc in the regulation of prostate cell growth.

Thus, in prostate cancer, 2 themes emerge from the analyses of zinc transporter expression in vivo: (1) the downregulation of zinc transporters by either genetic inheritance (African descent) or through aging (related to the modulations in the testosterone/prolactin levels or gene expressions acquired with old age) leads to the low accumulation of zinc in the prostate tissues,[12–19] and (2) the loss of the unique capability to retain normal intracellular levels of zinc caused by either the increased export or low import of zinc may be an important factor in the development and progression of malignant prostate cells.[1–5,10,29–35] From our data, it appears that the lowest degree of the expression of zinc transporters, *hZIP1* and *hZIP1*, is localized in the areas that exhibit neoplastic lesions, and is less dominant in the areas that are healthy-appearing. Our observation that there are differences in the zinc transport in different racial groups has great significance for prevention. If a role of zinc transporters is clearly established, then a zinc supplementation could be helpful in at least some people. Understanding the molecular events in the pathogenesis of prostate cancer is critical to the evaluation of the natural history of prostate cancer in humans, especially in various racial groups.[34–38] □

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2nd draft

MEDICAL HYPOTHESIS

Role of Zinc in Diabetic African Americans

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Inflammation and Diabetes Mellitus

Diabetes Mellitus (DM) is a life threatening disease because of the development of many of the severe secondary complications, including atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormalities, diabetic retinopathy, and ocular disorders. Generally, DM is classified as either an insulin-dependent type I or non-insulin-dependent type II DM. Type I DM is treated only by daily insulin injections; type II DM is treated by several types of synthetic therapeutic substances together with a controlled diet and physical exercise ().

Type I and type II diabetes are often thought of as diseases with completely distinct etiology. Thus, type I diabetes results from autoimmune destruction of insulin-producing pancreatic islet β-cells, while type II diabetes involves a constellation of metabolic disorders including insulin resistance, impaired control of hepatic glucose production, and β-cells dysfunction. A role for inflammatory mediators in the killing of β-cells in type I diabetes is clearly established. Destruction of β-cells results from direct contact with infiltrating T-cells and macrophages as well as exposure to inflammatory cytokines such as interferon (IFN)-γ, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and reactive oxygen/nitrogen species (ROS/RNS) that such cells produce (). More recently, inflammatory mediators have become increasingly implicated in the development of type II diabetes (). Key observations in support of this concept have included demonstration of a role for adipocyte-derived TNFα in the development of insulin resistance as well as studies showing that anti-inflammatory agents such as aspirin or salicylate are able to prevent or reverse insulin resistance via an effect on IκBα and NF-κB activation (). Moreover, studies in rodent models of type II diabetes such as the ZDF rat demonstrate an early compensatory increase in β-cell mass as insulin resistance develops, followed by a loss of β-cell mass as insulin secretion declines and frank diabetes ensues. That this loss of β-cell mass might be mediated by inflammatory agents is supported by studies in which lipid-laden islets of ZDF rats, or islets caused to store fat by chronic culture in fatty acids, are much more susceptible to cytokine-mediated cytotoxicity than islets from lean ZDF rats or normal islets cultured in the absence of fat, respectively (). It should also be noted that islets are thought to be more susceptible to damage from oxidative stress than other tissues due to their extremely low expression of oxygen radical metabolizing enzymes such as manganese superoxide dismutase (MnSOD), catalase, and glutathione peroxidase (). These findings provide motivation for studies on the mechanisms by which inflammatory agents cause functional impairment and cytotoxicity in β-cells, and also spur the development of methods for protecting islet cells against such attack ()

The biological relevance of the activation of inflammatory pathways became evident upon the demonstration that interference with these pathways improve or alleviate insulin resistance (). The abnormal production of (TNF-α) in obesity is a paradigm for the metabolic significance of this inflammatory response. When TNF-α activity is blocked in obesity, either biochemically or genetically, the result is improved insulin sensitivity (). Studies have since focused on the identification of additional inflammatory mediators critical in metabolic control and on understanding the molecular mechanisms by which inflammatory pathways are coupled to metabolic control. Recent years have seen a critical progress in this respect by the identification of several downstream mediators and signaling pathways, which provide the crosstalk between inflammatory and metabolic signaling (). These include the discovery of c-Jun N-terminal kinase (JNK) and I κβ kinase (I kappa K) as critical regulators of insulin action activated by TNF-α and other inflammatory and stress signals, and the identification of potential targets (2)

Cellular Zinc and Mechanisms of Action

Zinc is one of the most abundant nutritionally essential elements in the human body. It is required in nearly 300 enzymes (). It plays a catalytic, co-catalytic and/or structural role in the proper folding of proteins (). Zn-ATP is necessary for synthesis of pyridoxal-5-phosphate and flavin adenosine dinucleotide (FAD), coenzymes essential for biogenic amine synthesis and monoamine oxidase metabolism (). The role of zinc in protecting biological structures from damage by free radicals may be due to several factors: maintaining an adequate level of metallothioneins (MTs) (which are also free radical scavengers), as an essential component of superoxide dismutase (SOD), as a protective agent for thiols and in preventing the interaction between chemical groups with iron to form free radicals (). Zinc deficiency increases the levels of lipid peroxidation in mitochondrial and microsomal membranes. Its presence prevents lipid peroxidation (). Zinc is also an effector of tubulin polymerization and acts *in vitro* on actin filament formation and stabilization (). Hormonal regulation of zinc transport is best exemplified by the effects of insulin in mammary glands. Zinc finger motif is the most common recurring motif in transcription proteins (). The configuration of these "fingers" which determines their binding to DNA is determined by the single zinc atom at their base (). They initiate the transcription process and gene expression ().

Antioxidant Properties of Zinc

If an antioxidant is any substance that hinders a free radical reaction, then zinc which has never been shown to interact directly with an oxidant species does not fit such definition. Rather it exerts its effects in an indirect manner by stabilizing the cell membrane structure, contributing to the structure of the SOD and maintaining the metallothionein tissue concentrations. Antioxidants are both acute and chronic. Long-term deprivation of zinc renders an organism more susceptible to injury induced by a variety of oxidative stresses (). The acute effects involve two mechanisms: stabilization of sulfhydryls or reduction in the formation of OH from H_2O_2 and superoxide through the antagonism of redox-active transition metals such as iron and copper ().

Zinc and Regulation of Apoptosis

Increased apoptosis *in vivo* may occur as direct or indirect consequence of a decrease in intracellular zinc concentrations (). It was suggested that zinc may either block the mechanism by which the inactive procaspase-3 is processed and thereby activated or block the active caspase-3 cleaving its cellular substrates (). In support of these data, zinc supplementation suppressed a step before the activation of caspase-3, in colon cancer induced to undergo apoptosis by addition of the histone deacetylase inhibitor butyrate (). Caspase-6 is the most sensitive apoptosis-related molecular target of zinc. It is known to cleave and activate the proenzyme form of caspase-3 and is also responsible for the cleavage of lamins and therefore, is directly involved in nuclear membrane dissolution. It has been shown that Zn blocks these two events. Another cellular proteins that regulate a common pathway of apoptosis are the antiapoptoic Bcl-2-like and proapoptotic Bax-like mitochondrial membrane protein (). The ratio of Bcl-2-like to Bax-like proteins acts to determine in part survival or death of cells after an apoptotic stimulus (). Zinc supplementation of cells *in vitro* increased the Bcl-2/Bax ratio thereby increasing the resistance of the cells to apoptosis. The antioxidant properties of zinc may play a role in protecting the cell from oxidative stress. Increased apoptosis of thymocytes was due to excessive levels of circulating glucocorticoids triggered by a zinc-deficiency-associated stress response (). Microtubular skeleton which is disrupted in zinc deficiency and apoptosis was also induced in various types of cells when cultured in a zinc-free medium or zinc-depleted by membrane zinc-chelator (). Thus, increasing the intracellular zinc content inhibits colchicine-induced apoptosis. The use of a zinc-specific chelator, TPEN (*NNN'N'*-tetrakis- (2-pyridil-methyl) ethylenediamine) abolished the inhibition of apoptosis mediated by zinc (). Cells which have zinc pools and metallothionein can donate zinc to apoenzymes, synergize between zinc and glycoprotein (). Hence the dysregulation of cellular zinc during stress, aging and other may affect cell survival ().

Zinc Levels and Immune Status

Impairment of immune function has been attributed to zinc deficiency and may be the most common cause of secondary immunodeficiency states in humans (). Several studies have demonstrated that zinc inhibit phagocytosis, reducing the release of oxyradicals and basal production of superoxide and hydrogen peroxide (). The increase in ROS is apparently due to a G-protein coupled system activated by zinc ().

Zinc and Nitric Oxide (NO)

NO modulates inflammation and can act both as a proinflammatory and an antiinflammatory agent (). The anti-inflammatory activity of zinc was reported to be due to IL-1β induced NO formation and to the reduced activity of smooth muscle cell NO synthase and to lipopolysaccharide inhibition by endogenous zinc ().

Zinc deficiency is observed in alcoholic liver disease (ALD). ALD-related Zn deficiency is mediated by the proinflammatory cytokines, IL-1, TNFα and IL-8 as well as oxidative stress changes (). The intracellular transcription factor NFκB is also enhanced in the monocytes of patients with ALD as a consequence at least in part of oxidative stress. NFκB expression is reduced by the addition of zinc ions to mononuclear cells of ALD patients ().

In patients having esophagitis and were receiving H_2-receptor antagonists, esophageal tissue concentrations of zinc approached normal values (). In the stomach, zinc deficiency increases gastric secretory volume, acid and pepsin and promotes or aggravates stress-induced gastric lesions and decrease in mast cell count (). Thus, any state manifesting systemic zinc deficiency will promote or potentiate the development of gastric mucosal damage by increased mast cell histamine release and acid–pepsin secretion (3)

Metallothioneins

Metallothionein (MT) is a small metal-binding protein with 61–68 amino acid residues, which include 20 cysteines, that is bound to a total of seven equivalents of certain bivalent metal ions. MT can be induced in cells by metals, reactive oxygen species (ROS), hormones, cytokines, UV radiation, and alkylating agents. MT exists in four isoforms, MT-I through MT-IV. MT-I and -II are found in all types of tissues whereas MT-III is mainly expressed in brain cells and MT-IV is found in stratified squamous epithelium. Metal ions such as zinc and cadmium are robust inducers of MT and increase the transcription of MT by activating metal-regulatory transcription factor-1. Due to its abundant cysteine residues (25–30%), MT can bind metal ions with high affinity and has a role in metal detoxification and in the protection of cells against free radical injury. On the other hand, by removal and transfer of zinc, MT can modulate the activities of zinc-dependent regulatory proteins, including enzymes and zinc-finger transcription factors. Interestingly, several signaling molecules such as nitric oxide and oxidants have been reported to displace MT-bound zinc, allowing MT to participate in the mediation or augmentation of redox or NO signaling.

Strong pathophysiologic functional similarities exist between MT and NF-κB. Both are antiapoptotic entities especially in cancer cells and both have regulatory roles in inflammation. Moreover, many of the stimulators of their expressions overlap, i.e., tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS), and interleukin-1 (IL-1). Thus, it is plausible that there is a close molecular relationship between the two molecules. In fact, MT reportedly interacts with the p50 subunit of NF-κB to increase the transactivation of NF-κB. It was reported that an increase in MT expression attenuated the inhibitory effect of zinc on NF-κB activity in HeLa cells (4)

Zinc and Diabetic African Americans

African Americans disproportionately suffer from various diseases in the US. Many of these diseases include hypertension, lupus, cardiovascular disease, diabetes mellitus, and cancers of the prostate and pancreas. A number of risk factors such as smoking, a high fat diet, little physical activity, stress, and meager access to health care have been the subject of numerous investigations.

In the last half of the 20th century, investigations between the relationships among diseases and micronutrients, such as iron, copper, zinc, and selenium, have been numerous. Of note, the content of zinc in the pancreatic beta cell is among the highest in the body; however, little is known about the uptake and storage of zinc inside this cell. The high zinc requirement of the beta cell relates to each hexameric insulin crystal containing two zinc atoms in insulin granules. When exocytosis of insulin occurs, an insulin granule fuses with the beta cell plasma membrane and releases their contents into the circulation. Thus zinc is secreted along with insulin, thereby requiring the continual replenishment of the beta cell zinc. Because free zinc is toxic to a number of proteins in the beta cell, zinc must be complexed to the specific zinc binding proteins. These transport proteins play a role in zinc uptake and storage in pancreatic beta cells. Interestingly, according to some recent reports, the zinc concentration in patients with Type I diabetes is significantly lower than in healthy controls. These findings are not universal, and other researchers have found no significant differences in the zinc levels between DM and control subjects. However, in these studies racial groups were not studied in a separate manner.

Zinc and Prostate Cancer

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Please review the literature from our published article and attachment I am sending

Prostate Cancer and Zinc Transporters: In the United States, prostate cancer is the most commonly diagnosed male cancer and the second leading cause of all male cancer deaths. Furthermore, incidence rates are higher in African Americans than in any other racial group. Extensive research has shown that low serum levels of zinc are associated with the increased incidence of prostate cancer. We have evaluated 58 prostate cancer tissues in 2 major racial groups (30 from whites and 28 from African Americans) for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all 30 prostate cancer specimens obtained from white people, the degree of expression of these two zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients. We also found a significant down-regulation of these two zinc transporters in normal prostate tissues from African American men when compared with age-matched white men. The loss of the unique ability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. Our observation that the uptake of zinc may be different in racial groups is intriguing and relevant. Once these data are confirmed in larger groups, this finding could have a significant application as a preventive maneuver for at least for some people. Because dietary zinc supplements are relatively nontoxic, any efficacy trial would be low-risk ().

Zinc and Hypertension

See Attachment for review.

Hypertension and serum Zinc: African Americans have some of the highest rates of hypertension in the world. On the basis of our data related to prostate cancer, we hypothesize that a similar molecular mechanism in which certain enzymes that regulate blood pressure and are highly dependent on intracellular zinc levels may be responsible for hypertension (HT). We hypothesize that HT, DM, and prostate cancer may share a similar molecular pathogeneses. For example, arterial tone and water-electrolyte homeostasis are regulated by several peptides, including angiotensin II (AII), bradykinin (BK), atrial natriuretic peptide (ANP) and endothelins (ETs). Changing the concentrations of these peptides in the plasma, tissue, or urine by decreasing the levels of angiotensin II and endothelins, as well as increasing BK and ANP concentrations, is one way of modulating the hemodynamic load. Three enzymes essentially control the metabolism of these peptides: angiotensin-converting enzyme (ACE), neutral endopeptidase (NEP), and endothelin converting enzymes (ECE), all belonging to the group of zinc metallopeptidases. How do levels of intracellular or serum zinc levels relates to HT? By utilizing spontaneously hypertensive rats (SHR), Sato et al () fed a standard or a Zn-deficient diet for 4 weeks, and examined whether Zn deficiency affects systemic blood pressure (BP) levels in a genetically hypertensive state through a fall in the activity of Cu/Zn-superoxide dismutase (SOD). SHR that were fed a Zn-deficient diet had a progressive increase in systolic BP during the dietary conditioning. Consequently, SHR that were fed a Zn-deficient diet exhibited significantly increased levels of systolic BP by 2 weeks after the start of dietary treatment when compared with SHR fed a standard diet. Similarly, the levels of basal mean arterial pressure (MAP) indicated that at the end of the dietary treatment, SHR that were fed a Zn-deficient diet > SHR that were fed a standard diet. The administration of the nitric oxide synthase (NOS) inhibitor, L-NAME, caused an increase in the MAP levels in the two groups of rats, demonstrating the involvement of the vasodilator, nitric oxide (NO), in the regulation of systemic BP in a genetically hypertensive state. The expression of the endothelial (e) NOS mRNA and protein in the thoracic aorta paralleled basal MAP levels in the two groups of rats, suggesting the counter-regulation of eNOS against the developed hypertensive state in the SHR that were fed a Zn-deficient diet. On the other hand, the administration of the superoxide scavenger tempol (a SOD mimetic compound), led to a decrease in MAP levels in the two groups of rats, indicating the participation of the oxygen free radical, superoxide, in an increase in systemic BP in a genetically hypertensive state. As reported recently, the mechanism involved is likely due to a decrease in the action of the vasodilator, NO, based on the formation of peroxynitrite coming from the non-enzymatic reaction of superoxide and NO. In addition, tempol treatment completely restored MAP levels in SHR that were fed a Zn-deficient diet comparable to those observed in SHR that were fed a standard diet, indicating that a further increase in systemic BP levels seen in SHR that were fed a Zn-deficient vs. a standard diet is presumably brought by a reduction in the action of the vasodilator, NO, resulting from an increase in the action of superoxide. The activity of the superoxide scavenger, Cu/Zn-SOD, in the thoracic aorta was significantly decreased in SHR that were fed a Zn-deficient diet relative to SHR fed a standard diet. It appears that a decrease in the activity of Cu/Zn-SOD observed in the thoracic aorta of SHR that were fed a Zn-deficient diet at least in part plays a role in an increase in the action of superoxide in this model. Thus, Zn deficiency may be a factor to develop genetic hypertension presumably through the oxidative stress caused by superoxide.

The lack of a valid indicator precludes a true estimate of zinc deficiency in various hypertensive populations from different racial groups. However, Singh et al () have attempted to determine the association among current zinc intake, the prevalence of coronary artery disease (CAD), diabetes, as well as factors associated with insulin resistance. They studied 3575 subjects, aged 25 to 64 years, including 1769 rural (894 men, 875 women) and 1806 urban (904 men, 902 women) subjects. The survey methods included questionnaires for 7-day food intake record, physical examination, and electrocardiography using World Health Organization criteria. They determined that the prevalence of CAD, diabetes, and glucose intolerance was significantly higher among subjects consuming lower intakes of dietary zinc. There was a higher prevalence of hypertension, hypertriglyceridemia, and low high-density lipoprotein cholesterol levels that showed a significant upward trend with lower zinc intakes. Serum lipoprotein (a) and 2-hour plasma insulin levels also were associated with low zinc intake. Multivariate logistic regression analysis after adjustment for age showed that zinc intake and CAD were inversely associated. Serum zinc (odds ratio: men 0.77, women 0.57), serum triglycerides (men 0.86, women 0.81), blood pressure (0.83 men, women 0.76), diabetes mellitus (men 0.90, women 0.85), central obesity (men 0.88, women 0.87), glucose intolerance (men 0.66, women 0.57), and low high-density lipoprotein cholesterol (men 0.72, women 0.70) were significant risk factors for CAD (explained by tertiles of zinc status) in urban subjects. These associations were not observed in rural subjects. In summary they concluded that lower consumption of dietary zinc and low serum zinc levels were associated with an increased prevalence of CAD, diabetes, and several of their associated risk factors including hypertension, hypertriglyceridemia, and other factors suggestive of mild insulin resistance in urban subjects.

In one study, RT-PCR was used to examine the development of mRNAs encoding zinc transport proteins and other metal complexing proteins in pancreatic islets of the normal Sprague–Dawley rat and the BB diabetes resistant (BBDR) rat. The BB rat, which is derived from the Wistar strain, was studied primarily to provide a comparison with a rat strain dissimilar to the Sprague–Dawley strain, and secondarily to discern if there are differences between the BB strain and normal rats in the expression of islet proteins that might explain the immune destruction of the beta cell in the BB strain exhibiting a form of diabetes similar to human type 1 diabetes. The BBDR rat was selected because its beta cells are genetically identical to the BB diabetes prone rat (BBDP), but since only its immune system is different from the BBDP rat, its islets are not destroyed by an autoimmune attack [1] and are amenable to study. Delayed expression of a protein might cause an antigenic response against it due to the failure of immune self-tolerance [2 and 3] to properly develop against the protein if it appears abnormally late in development. A difference between the two strains of rats in the development of a protein might suggest a role for its acting as an antigen that initiates the autoimmune destruction of the beta cell.

The expression of genes encoding four zinc transport proteins ZnT-1, -2, -3, and -4, as well as calreticulin, ferritin, metallothionein 1, metallothionein 3, Nramp1, Nramp2, transferrin, and the transferrin receptor were studied with RT-PCR. As controls, the expression of genes encoding proteins known to be present in the beta cell plasma membrane (receptors for IGF-1, IGF-2 and insulin), cytosol (quinone reductase), microsomes (cytochrome b558), and the mitochondrial inner membrane (the tricarboxylic acid transporter) was studied. The results indicated that the mRNAs encoding all of the metal-complexing and control proteins studied were present at various stages of development. No major difference between the normal Sprague–Dawley rats and BB rats were observed. However, there were differences between the gene expressions in islets versus the whole pancreas in the adult animals. Therefore, RT-PCR was used to estimate the amounts of mRNAs encoding various metal-complexing proteins in the pancreas of the 3-day-old animals and islets from 10 days to adulthood in Sprague–Dawley and Wistar BBDR rats. The relative densities in agarose gels of the DNA bands generated from islets were compared with those from a control tissue chosen because it exhibits a high level of a particular protein. The expression of four genes, those encoding metallothionein 3, Nramp1, Nramp2, and ZnT-4, were not found in the three day old pancreas, but were present in islets from 10 days through adulthood. It is possible that the failure to detect the expression of these genes in 3 days is due to our using RNA from the whole pancreas to dilute the islet RNA. However, at age 3–6 days in the rat, islet volume is at its largest at any time in development and constitutes as much as 5% of the volume of the pancreas, whereas in older and adult animals, the islet volume is about 1% of the pancreas [5 and 6]. The genes encoding ZnT-2 and ZnT-3 were not expressed in the pancreas in either the 3-day-old or adult animals, but were present in islets of 10-day and 5-week-old animals.

The expression of the ZnT family of proteins has never been characterized in the pancreatic islet. ZnT-1 is a ubiquitous protein located in the plasma membrane; its purpose is believed to primarily export zinc from the cell [7] but the exact role of ZnT-1 in animal zinc homeostasis is unknown [8]. Although ZnT-2 and ZnT-3 are homologous to ZnT-1, they are more similar to each other than they are to ZnT-1. Both ZnT-2 and ZnT-3 are present in intracellular vesicles and are believed to be involved in cellular zinc transport, but their exact physiological functions are also unknown [9 and 10]. ZnT-2 has been shown to be present in the kidney, intestine, seminal vesicles, and testis [9]. ZnT-3 is expressed in the testis, parts of the brain, the cell bodies, and rich mossy zinc fiber projections of dentate granule cells of the hippocampus [10]. ZnT-4 is believed to be involved in either zinc efflux or compartmentalization, is abundant in mammary epithelia and brain, and is found in lesser amounts in a variety of tissues such as the liver, heart, and spleen. A nonsense mutation in the ZnT-4 gene is responsible for the condition in mice known as *lethal milk,* in which the decreased zinc transport into the mammary glands of mutant mothers causes zinc deficiency and death in nursing pups [11]. From birth to adulthood, islet volume increases more than 7-fold in the rat [6 and 12]. The presence of ZnT-2 and ZnT-3 mRNA in islets of 10-day and 5-week-old animals, but its absence in islets of adult animals may be a feature of rapidly developing islets. None of the known zinc transporters are believed to be responsible for the uptake of zinc into cells via its transport across the plasma membrane. Zinc uptake in the insulin cell probably occurs via the transferrin receptor that is known to transport zinc besides iron (see below).

The expression of the Nramp genes in islets has also never been studied. Nramp1 is an integral membrane protein of the lysosomes and endosomes expressed in the spleen and liver of mice, predominantly in the peripheral blood leukocytes of humans, and also in the lungs, liver, and spleen. In the mouse, susceptibility and resistance to infections such as Leishmania, Mycobacterium, and Salmonella is under genetic control of the Bcg/Ity/Lsh locus that codes for the Nramp1 gene [13]. Nramp1 and Nramp2 proteins share almost 80% sequence identity. Nramp2 (also known as DCT1) is a divalent cation transporter that is involved in protein coupled active ion transport that depends on membrane potential. It has a broad specificity including Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+}. Nramp2 is known to be expressed in the proximal intestine, kidney, thymus, and brain [14] and missense mutations in Nramp2 are known to cause the microcytic hyperchromic anemia seen in the mk mouse and the Belgrade rat [15 and 16]. Both Nramp1 and Nramp2 mRNA were absent in the 3-day-old pancreas, but Nramp1 mRNA was present at low to moderate levels in islets from 10 days to adulthood, Nramp2 mRNA was present at moderate levels at 10 days, and at high levels at 5 weeks of age and in adult islets (Fig. 1 and Table 2).

Calreticulin has been shown to be present in the exocrine pancreas, but little information is available about its expression in the endocrine pancreas. Calreticulin is a high affinity Ca^{2+} binding protein that has been shown to have many diverse roles including functioning as a molecular chaperone for insulin receptor monomers [17], and as a human rheumatic disease associated autoantigen [18]. Calreticulin is capable of binding zinc with a high affinity and low capacity [19], and zinc and cadmium increase calreticulin transcriptional activity [18]. Calreticulin mRNA was present at high levels in islets at all stages of development.

Metallothionein 1 is a well-known zinc complexing protein that has been reported to be constitutively present in mouse pancreatic islets [20], but the expression of metallothioneins 2 and 3 in islets has not been documented. All metallothioneins are small cysteine rich proteins that bind transition metals and are not involved in zinc transport across the plasma membrane. MT-1 is induced in islets by zinc, cadmium, streptozotocin, and functions as a zinc storage molecule and a free radical scavenger [20 and 21]. MT-3 differs from MT-1 and MT-2 in that it is not inducible by stimuli, such as metals, hormones, cytokines, and reactive oxygen species that normally cause the levels of these proteins to increase. MT-3 appears to be located primarily in the brain and to a lesser extent in the reproductive system [22]. MT-1 mRNA was present in the pancreas and islets at all ages studied. MT-3 mRNA was absent in the 3 day old pancreas, but present in 10 day, 5 week, and adult islets (Fig. 1 and Table 2).

Transferrin binds two ferric iron atoms and transports the iron between sites of absorption, storage, and usage [23]. Transferrin also binds zinc and may function in its absorption, transport, and cellular influx through the transferrin receptor route [24 and 25]. mRNAs encoding both transferrin and the transferrin receptors were present in the 3 day pancreas and in islets of 10 days, 5 weeks, and in adult rats (Fig. 1 and Table 2). The transferrin receptor (26 and MJM unpublished data) and ferritin have previously been shown to be present at high levels in islets of adult rats [27]. Since transferrin receptor gene expression is much higher in the islet than in the surrounding acinar tissue (Table 2), this may indicate that the transferrin receptor has an important role in the beta cell. Ferritin is a ubiquitous iron storage and transport protein that also binds metals such as Cu, Zn, Cd, Pb, Be, and Al, but not nearly in as large quantities as iron is bound [28]. mRNAs encoding both the light and heavy subunits of ferritin were present in islets at all ages studied. Since the amount of iron in the normal pancreatic islet is very low [26], this suggests that the transferrin receptor and ferritin function to complex a metal other than iron in the beta cell.

As controls, mRNAs that encode six other proteins known to be present in the beta cell and cytochrome b558, the receptors for IGF-1 [29], IGF-2 [30], and insulin [31], as well as quinone reductase [32] and the tricarboxylic acid transport protein were studied. While not all of these mRNAs were detected in the whole pancreas of 3-day-old and adult rats, they were all present at high levels in islets from 10 days to adulthood (Table 3).

Since there were no significant differences in the levels of expression of any of the genes studied in the pancreas or islets between the Sprague–Dawley rat and the Wistar BB rat, it is unlikely that any of the proteins encoded by these genes are responsible for initiating an autoimmune response due to the delayed expression of a protein in the beta cell causing a failure of immune tolerance to develop against the protein. The expression of a number of genes encoding proteins, including cytochrome b558, the IGF-2 receptor, the insulin receptor, quinone reductase, tricarboxylic acid transport protein, and many of the metal complexing proteins, was low or undetectable in the whole pancreas of the adult (Table 2 and Table 3) as expected from the contrasting metabolic functions between the endocrine and exocrine pancreas.

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Hypothesis

In the light of above discussion, we hypothesize that there is a significant difference in the degree of the expression of zinc transporters amongst various races. It is hypothesized that individuals of African descent may have a lower capacity to absorb zinc, or transport zinc inside beta cells, due to their inherent down regulation of zinc transporters, as opposed to other racial groups. We further hypothesize that as a result of zinc deficiency, pancreatic beta cells in African Americans are more prone to free radical injury with increased potential for development of DM, more so than White Americans. According to our hypothesis, the expression levels of inflammatory cytokines like TNF-a and NFkB is significantly lower and expression of NO should be significantly higher in pancreatic beta cells of African Americans as compared to White Americans.

Since Africa is a mineral-rich continent and the zinc levels in the water and diet are very high, Africans have genetically down-regulated their zinc absorption capacity; otherwise they would absorb abnormally higher levels of zinc, resulting in various serious neurodegenerative illnesses (5-6). Also, high levels of zinc have been known to cause a variety of other neurological and biochemical disorders (5-7). This down-regulation of zinc transporters can be compared with the down-regulation of melanin pigmentation in white individuals who have migrated to the tropics for several generations but cannot up-regulate their pigmentation to protect themselves from developing skin cancer due to solar UV light damage. Therefore it is hypothesized that when the African population entered Northern America, where zinc levels in the water and diet are relatively low, their inherent down-regulation of zinc absorption and/or transporters resulted in low intracellular zinc levels, subsequently resulting in the increased incidents of prostate and pancreatic cancers, DM, and perhaps hypertension (HT). Extensive research has shown that low serum levels of zinc have been associated with the increased incidence of prostate cancer and DM (8-9). This is because a normal human prostate and beta cells in the pancreas accumulates the highest levels of zinc in any soft tissue in the body. Long-term low serum zinc levels or the low capacity of prostate or beta cells to transport zinc inside the their cells may lead to a series of biochemical disturbances and molecular injuries, subsequently resulting in pancreas cancer. For example, the zinc level in prostate cancer is markedly decreased from the levels detected in normal pancreas tissues.

This hypothesis is based on current epidemiological data as well as on studies previously done by our lab. Incidence data indicate that DM rates are significantly higher for African Americans than for Caucasians or Asian Americans. African Americans have a higher prevalence of hypertension, diabetes, cardiovascular disease (CVD), stroke, prostate cancer, and renal disease than white Americans. The high rates of diabetes and hypertension in children of type 2 diabetes, stroke, and CVD in women are particularly striking. Our laboratory has been attempting to decipher the molecular mechanisms involved in the high incidence of development of prostate cancer in African Americans. We have evaluated prostate cancer tissues in the two major racial groups for their ability to express 2 major human zinc transporters, *hZIP1* and *hZIP2*. In all prostate cancer specimens obtained from white people, the degree of expression of these two zinc receptors was high when compared with age-matched and Gleason score-matched specimens obtained from African American patients. We also found a significant down-regulation of these two zinc transporters in normal prostate tissues from African American men when compared with age-matched white men. The loss of the unique ability to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. Our observation that the uptake of zinc may be different in racial groups is intriguing and relevant.

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